CLINICO-MICROBIOLOGICAL PROFILE OF *STAPHYLOCOCCUS AUREUS* PYODERMAS IN DERMATOLOGY OUTPATIENTS

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE RULES AND REGULATIONS FOR THE M.D. BRANCH XX DERMATOLOGY, VENEREOLOGY AND LEPROSY EXAMINATION OF THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY TO BE HELD IN APRIL, 2011

DEPARTMENT OF DERMATOLOGY, VENEREOLOGY AND LEPROSY
CHRISTIAN MEDICAL COLLEGE, VELLORE 632 004, S. INDIA.

Dr. Susanne A Pulimood,MD.,
Professor & Head
Outpatient clinic: Tues,Thurs,Sat 7.30am-11am
ID clinic: Wed 1.30-4.30pm
Cosmetology clinic: Wed 8am-12.30pm

Telegram :"MISSIONHOS"
Telephone:91-416-2283629/2282054

E.mail: derm@cmcvellore.ac.in
Fax 91-416-22232103 or 2232035

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30/9/2010

CERTIFICATE

This is to certify that the dissertation entitled "**Clinico-microbiological profile of *Staphylococcus aureus* in Dermatology outpatients"** is the bonafide original work of **Dr. Tanumay Raychaudhury.**

This study was undertaken at the **Christian Medical College and Hospital, Vellore** from the year 2009 under my direct guidance and supervision, in partial fulfillment of the requirement for the award of the **MD degree in Dermatology, Venereology and Leprosy of the Tamil Nadu Dr. M.G.R Medical University.**

GUIDE:

Dr. Renu George,
Professor,
Department of Dermatology,
Venereology and Leprosy,
Christian Medical College,
Vellore.

Head of the Department

Dr. Susanne A Pulimood,
Professor & Head,
Department of Dermatology
Venereology & Leprosy,
Christian Medical College,
Vellore.

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Head of the Department

Dr. Susanne A Pulimood,
Professor & Head
Department of Dermatology
Venereology & Leprosy,
Christian Medical College,
Vellore.

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INTRODUCTION

Interest in methicillin-resistant *Staphylococcus aureus* (MRSA), first discovered in 1961(1), stems from a number of factors, including the magnitude of the infections, concern over the development of antibiotic resistance, and versatility of the organism to produce multiple toxins leading to variety of clinical syndromes. It has drawn greater attention since a variant, community associated MRSA (CA-MRSA)(2) was first described. The original description and subsequent studies have noted that CA-MRSA is distinguished from health-care associated MRSA (HA-MRSA) by a more limited antibiotic-resistance profile, differences in toxins produced, the susceptible populations, and a propensity for outbreaks.(3) Skin and soft-tissue infections (SSTIs) represent the majority of the community-associated MRSA(CA-MRSA) disease burden, making the dermatologist the first in line(4) to detect the changing epidemiology of CA-MRSA. Infections caused by community-associated MRSA (CA-MRSA) differ from usual healthcare-associated MRSA (HA-MRSA) in their epidemiological, clinical and microbiological characteristics(5), and most importantly in the drug susceptibility profiles.

Since *S.aureus* is associated with significant morbidity(6) by causing primary and secondary pyodermas, the local epidemiological and microbiological understanding of this species is essential in appropriate health-care. Studies of MRSA have been carried out in different study populations, with incidence ranging from 1% to as high as 74%.(7)(8)(9) Studies from India are infrequent, with contrasting prevalence of MRSA in different geographical locations and among diverse populations.(10)(11)(12)

CA-MRSA and MSSA also harbour specific virulence genes associated with skin and soft-tissue infections, particularly the Panton–Valentine leucocidin (PVL) genes(13) and, occasionally, exfoliative toxin genes. PVL has been associated with higher recurrence, virulence, transmission and severity of SSTIs(14)(15)(16) and mainly linked to primary skin infections like abscesses, severe necrotic skin infections and furunculosis The PVL locus is suggested as a stable genetic marker of these CA-MRSA strains(14), which explains the frequency of primary skin infections associated with PVL, while there are conflicting data suggesting PVL to be only of epidemiological significance(4) and not to affect the final outcome even in complicated SSTIs.(9)

PVL-related infections further bear the risk of developing severe systemic infections such as bacterial endocarditis, necrotizing pneumonia and necrotizing fasciitis in both adults and children.(8)(17) Guidelines and algorithms for the management of PVL associated MRSA have been suggested in the UK(18)(19) due to higher prevalence and variable susceptibility to antibiotics. (5)(20)

The emergence of PVL positive isolates in community-associated staphylococcal pyodermas is globally described(21)(14), yet there were no reports of PVL associated pyodermas from the Indian subcontinent when this study was initiated. A descriptive prospective study on SSTIs would help in understanding the prevalence of CA-MRSA, guiding empiric therapy and also evaluate for the presence of PVL positive *S.aureus* in India.

Aims:

1. To study the clinical and microbiological features of *Staphylococcus aureus*, isolated from outpatients presenting with pyodermas to the Dermatology outpatient department between July 2009 to July 2010.

2. To compare the demographic and clinical features of methicillin-susceptible and methicillin-resistant *S.aureus* pyodermas

3. To determine the presence of PVL (Panton-Valentine leucocidin) gene in *S.aureus* isolates and study associated demographic and clinical factors

REVIEW OF LITERATURE

Staphylococcus (Greek: staphylē, "bunch of grapes" and kókkos, "granule") is a genus of Gram-positive bacteria. They are subclassified depending on their ability to produce coagulase. *Staphylococcus aureus* is coagulase-positive and catalase-positive, and is the most commonly isolated human bacterial pathogen.(22) It forms large, round, golden-yellow colonies on blood agar, the etymological root of the species, aureus meaning "golden" in Latin.

S.aureus is an important cause of skin and soft-tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis.(23) Methicillin-resistant *S.aureus* (MRSA) isolates are resistant to all available penicillins and other β-lactam antimicrobial drugs.(24)

MRSA had been considered a nosocomial pathogen since its first description in the early 1960s.(1) There have been increasing reports of community associated isolates of MRSA.(15) SSTIs represent the majority of the community associated MRSA(CA-MRSA) disease burden, which means that dermatologists are first in line to pick up a changing epidemiology of MRSA.(5)(4) Infections caused by CA-MRSA differ from usual healthcare-associated MRSA (HA-MRSA) in their epidemiological, clinical, microbiological characteristics(5) and most importantly in the drug susceptibility profiles.

Importance of *Staphylococcus aureus* in pyodermas:

Cutaneous pyodermas constitute a major burden of preventable disease in dermatology outpatients.(16) A significant number of these pyodermas are caused by bacteriae, among

which *S.aureus* is one of the most commonly implicated organisms in developed countries(20) and in India.(25)

Table 1.Prevalence of bacterial isolates from pyodermas(20)(25)

	USA (n=26233)	France {n=23012)	Germany (n=17205)	Italy (n=6884)	Spain (n=11764)	India (n=2783)
S.aureus	23.7	29.2	18.8	24.2	17.0	38.1
CoNS	11.1	13.5	11.8	12.6	13.1	5.5
Enterococcus	24.9	10.3	16.0	19.7	18.1	2.2
E.coli	8.8	9.9	14.5	7.8	12.7	17.4
P. aeruginosa	8.7	8.9	5.3	16.1	8.4	11.8
Klebsiella	5.0	2.8	4.4	2.5	3.9	6.7
Enterobacter	3.9	3.7	2.8	3.2	3.9	2.8
Proteus	2.9	4.6	3.6	3.8	4.6	2.3
Citrobacter	1.7	0.9	1.6	1.3	3.9	2.9
Serratia	1.5	1.3	0.7	0.7	2.3	NA
Acinetobacter	1.5	1.0	1.1	0.2	1.1	10.2
VGS	1.4	2.1	1.4	1.3	4.8	0.2
Bacteroides	0.5	2.0	6.0	0.8	0.9	NA
S.agalactiae	0.4	1.0	0.8	0.4	0.9	NA
S.pyogenes	0.2	0.7	0.5	1.0	0.4	0.2
Other species	3.8	8.2	10.7	4.6	6.8	0.7

CoNS: Coagulase Negative Staphylococci; VGS: Viridans Group Streptococci

The normal skin is colonized by commensal bacteriae that live on the surface and within the follicles.(26) The organism, species, its abilities for virulence, number of organisms in the inoculum and even strain differences in the same species may be important in being pathogenic or non-pathogenic resident commensal flora.(27)

The coagulase-positive Staphylococcus aureus, should not be considered as a resident on healthy skin in most subjects, although it frequents the anterior nares and perineal skin in one-third of the population. The other sites of carriage are the axillary skin, toe clefts and hands.(26)

EMERGENCE OF MRSA AND LEUCOCIDIN:

In 1961, soon after the introduction of methicillin, the first β-lactamase-resistant penicillin, strains of *S.aureus* that were resistant to methicillin were identified in the United Kingdom.(1) Prior to the mid-1990s, investigation into the epidemiology of MRSA was limited largely to the health care setting because it was rare that MRSA strains would infect otherwise healthy people.(28) The recognized risk factors then identified for MRSA infection and colonization included recent hospitalization; other exposures to the health care system; residence in a long-term care facility(29), acute-rehabilitation unit(30); the presence of an indwelling line or catheter; surgical wounds; chronic liver, lung, or vascular disease; malignancy; recent exposure to antibiotics; intravenous drug use(29); and exposure to a patient with any of these risk factors for MRSA.(31) From the early 1990s onward, MRSA infections were reported in otherwise healthy young individuals, and the respective strains named “community-associated MRSA”(CA-MRSA).(32)(33) CA-MRSA is distinct from HA-MRSA.(34) HA-MRSA

corresponds to definite predominant clonal lineages of the *S.aureus* population with some of them having pandemic dissemination (35), whereas CA-MRSA strains usually represent different lineages. Owing to the confusion concerning clear definitions of MRSA detected outside the hospital setting, there are several limitations of the current data on CA-MRSA.(36)

Although the pathogenicity and virulence of *S.aureus* is related to various surface proteins, epidemiological data revealed that synergohymenotropic toxins, also called leucocidin, described by Panton et al, is preferentially linked to furuncles, cutaneous abscesses and severe necrotic infections.(7)(37)(38)

Lina et al(8) demonstrated 93% Panton Valentine leucocidin(PVL) positive isolates in community acquired *S.aureus* in furuncles and 85% in severe necrotic hemorrhagic pneumonia, from clinical samples collected between 1985 till 1998 in Lyon, France.

While there were multiple prospective studies in Europe in France, UK and Germany, the first clinical isolate known to carry the PVL genes from Asia was reported in 2003.(39) In India, reports of MRSA have been present since 1980s although the major studies appeared in early 1990's, including surveillance studies and multicentric analysis in 1996.(40)(41) The first report of PVL however was only in 2009.(17) PVL harboring *S.aureus* have been suggested in large numbers in India from a multicentric trial on retapamulin.(21)

Emergence of MRSA in dermatology outpatients

Since the most frequent of infections caused by *S.aureus* are SSTI's, it is only obvious that cutaneous infections by staphylococcus would indicate the first changes in epidemiology and the resistance patterns**.** Until 1987 no MRSA was isolated in a dermatology outpatient clinic(42), whereas a gradual increase in infections with MRSA from 1.5% of all *S.aureus* in 1988 to 11.9% in 1996 in the same dermatology outpatient facilities was observed, and the prevalence is still increasing.(43) McBride et al.(42) studied 116 isolates from 2 different dermatology outpatients in Houston, Texas, USA and found none of the isolates to be methicillin-resistant. Uchizono et al.(44) reported MRSA strains in Japan in 1990 from various departments at the Kagoshima University Hospital, including the dermatology outpatient department.

In a randomised, double-blinded, placebo-controlled trial with cephalexin for uncomplicated cutaneous pyodermas in 2007 (45), *S.aureus* was isolated from 70.4% of abscess cultures. Of the isolates tested, 87.8% were MRSA, 93% of which were positive for PVL genes. This study demonstrated the extremely high prevalence of CA-MRSA associated with PVL.

There are totally seven published Indian studies that have looked into cutaneous pyodermas as of date.(11)(12)(46) There was a report of 10 cases of MRSA isolation in a dermatology inpatient setting in Mumbai in 2003.(46) These patients were inpatients who were found to have MRSA on wet lesions, which were swabbed, indicating possible primary or secondary infection or colonization. Retrospective analysis of pus swabs collected from hospitalized patients in New Delhi in 2004 showed a high prevalence of

38.6% of MRSA.(25) This prevalence of MRSA was noted to be higher than elsewhere in India, from earlier studies from Nagpur(47) and Vellore.(40) All these studies are done on clinical specimens from in-patients. These isolates might include HA-MRSA and hence may not be representative of the community prevalence of MRSA associated with SSTIs.

MRSA is reported from general population cohorts in dermatology outpatient departments from Mangalore(11) and Mumbai.(12) However, both studies showed variable prevalences of 11% and 0.9% respectively of MRSA in pyodermas from Indian patients. In a pediatric cohort in New Delhi, Sardana et al. (48) noted 6.9% prevalence of MRSA. Thind et al. (49) recently reported a prevalence of 9.6% of MRSA in a dermatology outpatient from a tertiary care hospital in New Delhi in 2010. While these studies showed a relatively low prevalence of MRSA in the Indian setting, conflicting data is presented in a study from the general population elsewhere in North India from Chandigarh with MRSA prevalence being 23.08%.(50)

In another retrospective analysis, Shenoy et al(51) described 83 CA-MRSA strains isolated from SSTIs from Mangalore. This retrospective study carried out by the microbiology department did not provide data on the prevalence on MRSA and discussed the antimicrobial susceptibility profiles of CA-MRSA in isolates from SSTIs, suggesting a variable antimicrobial susceptibility in CA-MRSA as compared to HA-MRSA. Thind et al(49) also showed that CA-MRSA are more likely to be non-multi resistant oxacillin resistant *Staphylococcus aureus* (NORSA), allowing more therapeutic options for treatment.

The prevalence of MRSA in Indian patients, especially in different geographic centres, urban or rural, are probable to vary, but there is dearth of data as to guide empiric therapy or help establish guidelines for management. In the event of MRSA prevalence being greater than 10%, a culture may be necessary, if clinically indicated, for appropriate therapy.(19)

Community associated MRSA

The terms CA-MRSA and HA-MRSA have been used to call attention both to the genotypic differences of certain MRSA isolates as well as to the epidemiological and clinical features of the pyodermas that they cause. Jappe et al.(4) has tried to delineate these differences in his study on cutaneous pyodermas, in similar lines as Del Guidice et al.(5) An essential component of epidemiological studies has been to define the clinical burden of CA-MRSA and HA-MRSA isolates, both of which circulate in the community. Important concepts bearing on these definitions are (i) the setting in which the MRSA infection begins; (ii) current or prior patient exposure to health care settings; (iii) poorly defined CA-MRSA patient risk factors, including prior MRSA infection; (iv) genetic characteristics and antibiotic susceptibilities of the causative MRSA isolate; and (v) the clinical syndrome manifested by the patient. (4)(15)

Case definition of CA MRSA

In 2000, the CDC created a case definition for a CA-MRSA infection: any MRSA infection diagnosed for an outpatient or within 48 hours of hospitalization if the patient lacks the following health care-associated MRSA risk factors: hemodialysis, surgery,

residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient.(52) All other MRSA infections were considered to be HA-MRSA. This case definition was initially used to demonstrate that MRSA infections were occurring among healthy people in the community without health care exposure.(53)

Table 2. Differences between HA-MRSA and CA-MRSA (19)

Parameter	HA-MRSA	CA-MRSA
Typical patient	elderly, debilitated and/or critically or chronically ill	young, healthy people; students, professional athletes and military service personnel
Infection site	often bacteraemia with no obvious infection focus. Also surgical wounds, open ulcers, IV lines and catheter urines. May cause ventilator associated pneumonia	predilection for skin and soft tissue, producing cellulitis and abscesses. May cause necrotising community acquired pneumonia, septic shock or bone and joint infections
Transmission	within healthcare settings; little spread among household contacts	community-acquired. May spread in families and sports teams
Clinical setting of diagnosis	in an inpatient setting, but increasingly HA-MRSA infections in soft tissue and urine are occurring in primary care	in an outpatient or community setting
Medical history	history of MRSA colonization, infection, recent surgery; admission to a hospital or nursing home, antibiotic use; dialysis, permanent indwelling catheter	no significant medical history or healthcare contact
Virulence of infecting strain	Community spread is limited, PVL genes usually absent	community spread occurs easily. PVL genes often present, predisposing to necrotising soft tissue or lung infection
Antibiotic susceptibility	often multiresistant with result that choice of agents often very limited	generally susceptible to more antibiotics than HA-MRSA

PVL and CA-MRSA infections

PVL is a two-component *S.aureus* pore-forming protein encoded by the lukF-PV and lukS-PV genes. It was first described in 1894 by Van de Velde and was associated with SSTIs in 1932 by Panton.(13) The genes encoding PVL, which can spread from strain to strain by bacteriophages, were previously believed to be present in fewer than 5% of unselected clinical *S.aureus* isolates before the advent of CA-MRSA strains in the mid-1990s, although the genes were transiently found in a circulating ST30 clone in Japan in 1979 to 1985.(54)

PVL is a bi-component (lukS-PV and lukF-PV) pore forming exotoxin that targets cells of the immune system such as polymorphonuclear cells.(55) PVL has been associated with higher recurrence, virulence, transmission and severity of SSTIs (skin and soft tissue infection) and mainly linked to primary skin infections like abscesses, severe necrotic skin infections and furunculosis.(4) The prevalence of PVL in *S.aureus* can be as high as 55-93% isolates from primary cutaneous infections. (4)(5)(8)(56)(57) PVL-related infections further bear the risk of developing severe systemic infections such as bacterial endocarditis, necrotizing pneumonia and necrotizing fasciitis in both adults and children.(8)(22) (58)

An intercontinental study with isolates from North America, Europe and Australasia showed CA-MRSA strains to share a type IV SCCmec cassette and the PVL locus, whereas the distribution of the other toxin genes was quite specific to the strains from each continent.(14) The PVL locus represents a stable genetic marker of these CA-MRSA strains.

Guidelines and algorithms for the management of PVL associated MRSA have been suggested in the UK(19), while there are no prospective studies targeted at PVL associated staphylococcal SSTIs from India till date.

In the United States, carriage of the PVL genes has been closely linked to infections caused by CA-MRSA strains in numerous epidemiological studies. In 2000, a large study from Minnesota found that 77% of patients with infections caused by CA-MRSA isolates (by the CDC case definition) were PVL^+, but only 4% of HA-MRSA isolates were PVL^+ .(3) Among 812 military recruits in Texas in 2003, 66% of 45 MRSA strains colonizing the nares of recruits or causing infections among them were PVL^+.(59) Among MRSA isolates from detainees in the San Francisco County Jail, more than 70% were PVL^+; of MRSA isolates from a clinic specializing in the treatment of SSTIs (all collected in 2000), 69% were PVL^+. All PVL^+ MRSA strains also carried the SCCmec type IV element.(60)

While PVL has been strongly linked epidemiologically to prevalent CA-MRSA strains, it is not known with certainty how they contribute to their fitness and/or virulence or if they are merely a marker for other fitness or virulence determinants. PVL^+ strains carrying SCCmec type IV, V, or V_T with varied background genotypes in many geographic settings have now been identified, although the chromosomal site of PVL gene integration lacks any known genetic linkage to the insertion site of SCCmec elements. Moreover, no other *S.aureus* toxin genes have been associated as strongly with CA-MRSA strains as PVL.(14) PVL genes are rarely found in MRSA strains carrying SCCmec types I, II, and III. There are reports of PVL associated with V_T strains from Taiwan in children with SSTI and atopic dermatitis.(61)

In the United States, PVL genes have been almost universally detected among CA-MRSA strains causing SSTIs.(62) In Queensland, Australia, in 2004 to 2005, 59% of SSTIs caused by PVL^+ *S.aureus* strains were designated "furunculosis," compared with only 10% of SSTIs caused by PVL-negative strains.(63)

The first prospective study on the emergence of MRSA in dermatology patients was conducted in France(5), where they found 11% MRSA in strains collected between 1999-2003. Of these 11%(22 isolates), CA-MRSA accounted for 27% of these, 100% being positive for PVL. Del Guidice noted a greater severity of the clinical cases (abscesses/furuncles) associated with CA-MRSA necessitating surgical incision and drainage in most cases. This was in contrast to 77% positive CA-MRSA isolates described by Naimi et al(3) in 2003 although the clinical severity of skin infections were not described. Sardana et al(48) reported a low prevalence (6.7%) of MRSA in a pediatric cohort from North India, in response to this study, suggesting that probably the age cohort and the geographical locations were important.

In the largest prospective study so far done on cutaneous pyodermas directed at PVL+ isolates at the University of Heidelberg, Germany, Jappe et al(4) noted 20/36 patients with deep skin infections having PVL-positive strains (55% of furuncles and 56% of abscesses). Similarly designed studies had revealed results on PVL-positive strains from deep skin infections with rates between 35.2 and 70.8%.(56)(57) The study also indicated that not everyone with invasive *S.aureus* infections carries PVL-positive (methicillin-resistant) strains, which contradicts the significance of PVL positivity as a *conditio sine qua non* (7)(8) for the development of deep skin infection.

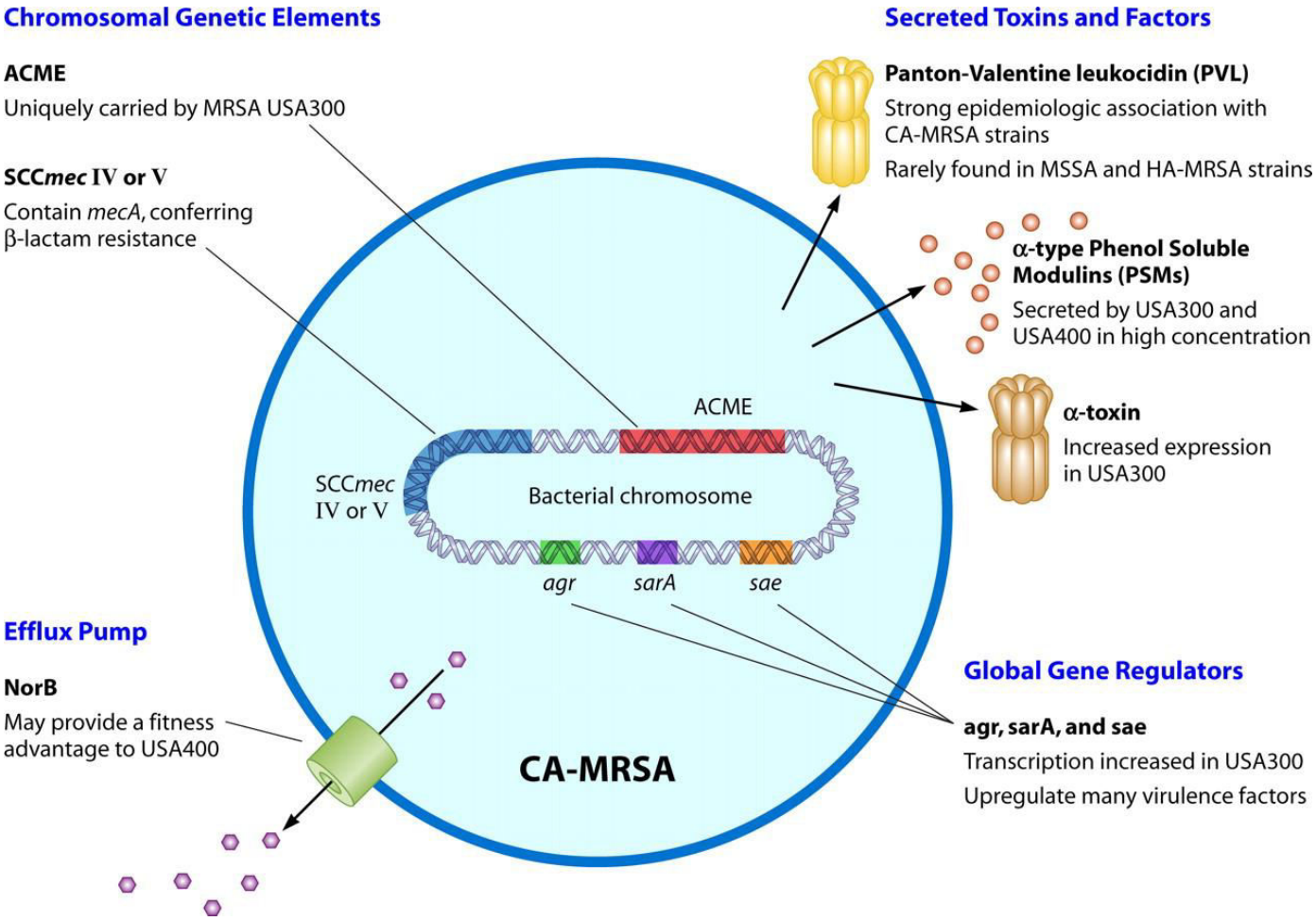


Figure 1. Schematic diagram of key proteins associated with resistance and virulence in CA-MRSA (Adapted from David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin. Microbiol. Rev. 2010 Jul;23(3):616-687.)

In Asia, the reported occurrence of MRSA infections that have onset in the community and the rate of PVL gene carriage have varied by country. In Taiwan, PVL^+ CA-MRSA strains of a single background type commonly cause infections. PVL genes were identified in all 17 isolates in one study of CA-MRSA infections in children, the majority of whom had SSTIs.(64)

In South Korea and China, PVL^+ strains remain rare among reported community-onset MRSA infections. Only 1 of 138 MRSA isolates from patients in South Korea with CA-MRSA infections (by CDC criteria) in 2004 to 2005 was PVL^+.(65)

The first published report of PVL positive strain from India was described in a MRSA isolate from Chennai, where a 13 year old boy had developed invasive septicemia and respiratory distress requiring acute medical care, the initial symptoms being a boil with fever.(17)

Emergence of PVL positive isolates in CAMRSA and HAMRSA has been reported from Chennai, India in 2009.(66) PVL positive strains have also been described from nasal swabs of epidemic MRSA in patients with SSTIs in Bangalore, India(67) in 2010 and from random isolates in MSSA and MRSA isolates from miscellaneous collections in Mumbai.(68)

In a study on molecular epidemiology of MRSA and MSSA isolates from global clinical trials(21), isolates were recovered from patients with uncomplicated skin infections in 10 different countries during five phase III global clinical trials of retapamulin, a new topical antibiotic agent. This study revealed a potentially disturbing finding that there were large numbers of PVL-positive MSSA isolates collected in South Africa and India. While the frequency of PVL-positive MSSA is generally considered to be low around the world, this study, in agreement with other recent work by Bae et al(9), provides evidence that some regions may have exceptional high levels of PVL-positive isolates. The data from India remains grossly underrated probably because there have been hardly any studies

directed towards the same. Bae et al(9) demonstrated that in complicated SSTIs, the presence of PVL did not alter the outcome of disease.

Role of PVL in the pathogenesis of MRSA infections: the "spider-bite"

PVL is a leukocidin that can lyse the cell membrane of human neutrophils, although its importance in pathogenesis is controversial. Recent evidence suggests that PVL may also inactivate mitochondria (69) and culminate in apoptosis. In animal models, PVL has been shown to be dermonecrotic(70), perhaps explaining the pathobiology of the characteristic skin lesions associated with CA-MRSA SSTIs. The lesions often resemble an injury produced by a spider bite(71), although common house spiders were not found to be carriers of CA-MRSA.(72)

Known risk factors for CA-MRSA (22):

Neonates Children beyond the neonatal period Athletes Household contacts of MRSA SSTI patients Emergency department patients Urban underserved communities	Detainees in jail or prison Cystic fibrosis patients Military personnel Men who have sex with men HIV patients Veterinarians, livestock handlers, and pet owners Indigenous populations

Neonatal MRSA Infections

Several neonatal MRSA outbreaks have been linked to CA-MRSA strains have been associated with visiting fathers, maternal mastitis, expressed breast milk, peripartum maternal MRSA infection, and health care workers colonized or infected with MRSA, implying a community source.(73)

Vertical transmission undoubtedly plays a role. There is evidence that vaginal colonization with *S.aureus* is common (74), but vaginal MRSA colonization is unusual and has rarely been linked to neonatal infection. In New York City in 2005, 0.47% of 2,963 pregnant women undergoing culture for group B streptococcal vaginal colonization had vaginal MRSA colonization.(75)

Colonization in the hospital by means other than vertical transmission likely plays a major role in neonatal colonization. Colonization of mothers was associated with black race, antibiotic use during pregnancy or intrapartum, and attendance by another child of the mother at a day care or an after-school program.(76)

In Japan, oropharyngeal MRSA colonization in the first week of life in extremely low-birth weight infants in an intensive care unit in 1997 to 2003 was associated with an increased risk of MRSA sepsis and with MRSA colonization during week 6 of life. Those authors hypothesized that colonization of the oropharynx in the first week of life with other, nonpathogenic bacteria may protect against colonization with MRSA.(77)

Children beyond neonatal period

Children who presented in 2002 to 2003 to Johns Hopkins University Hospital with a skin infection caused by *S.aureus* were more likely to have an MRSA infection if they were African American or if they lived near the hospital, an area with a high rate of poverty.(75) Of *S.aureus* SSTIs in children at the Johns Hopkins ED in 2003 to 2005, 73% (217/296) were caused by MRSA; 81% were CA-MRSA infections (by the CDC criteria) in 2003, and this percentage increased to 85% in the second year.(78)

In Asia, reports that identified isolates from children were predominantly ST59 and PVL^+ and carried SCCmec type V or V_T have come from Taiwan, South Korea, and Japan.(61)

In Buenos Aires, Argentina, in 2005, five SSTIs in children without exposure to the health care system were caused by PVL^+ MRSA strains carrying SCCmec type IV.(79) At a clinic in Athens, Greece, 88 CA-MRSA infections (by CDC criteria) among children younger than 14 years old were recorded in 2003 to 2005; 68% (28/41) of the strains belonged to one PFGE clonotype (similar to the PFGE type of ST80 strains) that was PVL^+ and SCCmec type IV. Twenty of the 28 strains (71%) belonging to this clonotype were isolated from patients with SSTIs.(80) The study from University of Heidelberg, Germany showed a higher risk of CA-MRSA and PVL carriage in younger individuals.(4)

In Narketpally, Andra Pradesh, India, in 2006, 12/392 (3.1%) children aged 5 to 15 years had nasal carriage of MRSA, but genotyping studies were not conducted.(81) In New Delhi, 3/89 (3.4%) of all isolates from pyodermas in children below 12 years of age were found to have MRSA.(48)

Thus, CA-MRSA infections and carriage have been reported across the world including India; pediatric infections may be a harbinger of an epidemic to come in the general population.(82)

Athletes

While MRSA SSTIs have afflicted participants in many sports, football teams(American football) have been most frequently implicated. In a meta-analysis of players from three

division I college football teams in 2003 to 2006, MRSA infections occurred in 6.7% (33/491) players, primarily on the extremities, with no relationship to position played.(83)

Household contacts of MRSA SSTIs

In Europe, where contact tracing of index MRSA cases is commonly performed, several case reports demonstrated household transmission.(84) In Greece in 2003 to 2005, among 88 CA-MRSA infections of children, 15.9% had suspected transmission of MRSA from family members.(80)

Reports of household contact transmission of CA-MRSA have been documented among health care workers(85) and by heterosexual spread(86), besides fomites as a cause for spread in patients with SSTIs.

Low socioeconomic status and overcrowding

Although there is no direct evidence for the same, the high prevalence of CA-MRSA among emergency departments (EDs) in the USA may index towards the same. EDs serve as a safety net that provides health care for uninsured populations, and thus, the number of SSTIs treated may reflect the prevalence of these infections in communities with a low socioeconomic status (SES).(87)

Cultured skin lesions among adults presenting to an ED in Cincinnati with an SSTI in 3 months during 2005 yielded MRSA at a rate of 58%. Risk factors for MRSA infection included in a best-fit multivariable regression model were young age, sexual contact in the past month, the presence of an abscess cavity, and residence in a group home(88).

Incarcerated populations, like in prisons and jails have also been found to have high prevalence of MRSA, isolated from SSTIs. Poor hygiene, fomite spread, prior antibiotic use, trauma and self-draining of boils have been identified as risk factors in this distinct population.(89)

Military personnel have a higher risk of transient colonization and SSTIs secondary to CAMRSA. Among active military service members deployed to Iraq in March to July 2008, 66 were diagnosed with a carbuncle, furuncle, boil, or abscess, as determined by a retrospective review of administrative records, of which 68% culture-positive isolates were MRSA.(90)

Medical comorbidities

Higher risk of MRSA colonization is well described on the skin in atopic dermatitis, diabetes mellitus, chronic erosions, venous leg ulcers, hemodialysis and cystic fibrosis.(4)

Men who have sex with men

At a clinic in Boston where approximately 70% of patients were MSM by self-report, 3.8% (30/795) of patients in 2005 to 2007 had MRSA recovered from the nares, the perianal region, or a skin infection. Among enrollees, 3.7% (29/795) had an SSTI at enrollment, and 9.2% (73/795) of patients had an SSTI during a 16-month period in 2005 to 2007. The authors of that study suggested that skin-to-skin contact and multiple sexual partners may predispose one to SSTIs caused by MRSA.(91)

Outside the United States, studies have not shown an elevated rate of MRSA carriage in MSM.

Clinical predictors of a pyoderma caused by MRSA

Morphology. Purulent SSTIs caused by CA-MRSA strains are the most common clinical manifestations of CA-MRSA.

An uncomplicated CA-MRSA SSTI typically presents as an abscess that may resemble a spider bite filled with purulent material.(22) This lesions present as spontaneously appearing raised tender red lesion, which may progress to develop a necrotic centre. This may lead to the suspicion of a 'spider bite' where such occurrences are common, e.g. North America or Australia. Most reports of such lesions have come from the USA and have not been as frequently reported from other countries. In the UK, where spider bites are rare, these 'dermatonecrotic' lesions increase the likelihood of a diagnosis of CA-MRSA but are not pathognomic. They can also be found in infections due to PVL-positive MSSA strains.

Among adults with an abscess and surrounding erythema, the presence of a central black eschar had a positive predictive value of 94% and a negative predictive value of 45% for an MRSA isolate.(92)

However, SSTIs can vary in appearance and can also present as folliculitis, paronychia, furuncle, felon, cellulitis with drainage (93) , or lymphadenitis.(94) Many other severe cutaneous complications of CA-MRSA have been reported and include extensive cellulitis, necrotizing fasciitis and purpura fulminans. Involvements of adjacent structures, either by direct spread or bacteraemia, such as septic thrombophlebitis, pyomyositis, septic arthritis and osteomyelitis, has all been described.

The spectrum of disease caused by CA-MRSA appears to be similar to that caused by CA-MSSA. Furuncles, carbuncles and other abscesses appear to be the most frequently

reported clinical manifestations. Erythematous papules and nodules, folliculitis and/or impetigo are less common presentations of CA-MRSA.(19)

No particular patterns of clinical presentation have yet emerged to allow differentiation from MSSA infections. Anecdotal reports suggest that recurrent (two or more in 6 months) furuncles or abscesses, or clusters of infections within a household may indicate PVL-positive CA-MRSA. However, this pattern can also be seen in PVL-positive MSSA infections. Hence, except for the distinctive appearance noted above, MRSA SSTIs cannot be distinguished from SSTIs caused by other agents, including MSSA(95) on clinical grounds.

Location. CA-MRSA abscesses can be found in diverse anatomical locations, including the breast(96), vulva(97), hand after clenched-fist injury(98), and neck.(94)

Recurrence. Small studies have suggested that the recurrence of CA-MRSA SSTIs is common after treatment. A recurrence of 1 to 3 CA-MRSA (i.e., onset outside the health care setting) SSTIs at a distinct anatomical site occurred among 5 of 11 adult HIV-infected patients in Chicago in 2003 to 2004; the intervals between index lesions and recurrences were not stated.(99) Among 87 MSM in New York City who presented with a CA-MRSA (i.e., onset in the community) SSTI, 31% had a recurrence within 6 months after the resolution of the initial infection. Recurrence rates among those receiving MRSA-appropriate and MRSA-inappropriate initial antibiotic therapy (21/63 versus 6/20) were similar.(100)

The likelihood of recurrence was not significantly different among HIV-infected patients, recipients of TMP-SMX prophylaxis, individuals with MRSA nasal colonization, or recipients of mupirocin for nasal decolonization.(101)

Recurrence of skin infections and household clusters has been included in the risk-factor group for acquiring MRSA infection.(22), as is supported by studies both from developing (102) and developed countries.(16)(103)

MATERIALS AND METHODS

Study design: Open, prospective study with cross-sectional study design

Study setting: This study was conducted at the outpatient department of Department of Dermatology, Venereology and Leprosy in Christian Medical College and Hospital, Vellore.

Study subjects:

Eligibility: All patients presenting to the outpatient department with primary or secondary pyodermas were eligible for the study.

Inclusion criteria:

- All patients presenting to Dermatology OPD with pyoderma (primary or secondary) and willing to participate in the study

Exclusion criteria:

- Patient who has been partially or completely treated for the current episode of pyoderma with antibiotics with improvement of symptoms
- Patient presenting with lesions classically known to be caused by non-staphylococcal organisms
- Patient presenting with staphylococcal pyodermas known to be caused by toxins such as Staphylococcal Scalded Skin Syndrome etc.

Study period: July 2009 to July 2010

Research committee approval: The study was approved by the Institutional Review Board and Ethics Committee.

Methodology:

All patients with pyoderma who were eligible for the study after meeting the inclusion and exclusion criteria were asked to participate in the study.

Case-definitions, definitions of demographic variables and definitions of clinical syndromes were used as given below:

Primary pyoderma: Primary infections were those occurring on apparently normal skin. The diagnosis of primary infection was based on clinical findings as described. (27)

Secondary pyodermas were those arising in damaged skin (traumatized skin, or a pre-existing skin disease. (5)

Recurrent pyoderma: 2 or more episodes of pyoderma, distinct from the index pyoderma, noted more than 6 days apart, over past 6 months. (99)

Disseminated infection: 2 or more non-contiguous body sites with pyoderma (23)

MRSA (methicillin-resistant *S.aureus*) (49): Strains of *S.aureus* that are resistant to the isoxazoyl penicillins such as methicillin, oxacillin and flucloxacillin. **MRSA** are cross-resistant to all currently licensed β-lactam antibiotics.

CA-MRSA:

Any MRSA infection diagnosed for an outpatient or within 48 hours of hospitalization if the patient lacks the following health care-associated MRSA risk factors: hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient.

HA-MRSA:

All the other MRSA isolated from inpatients after 48 hours of hospitalization or with any of the above mentioned risk-factors were considered to be HA-MRSA.

Clinical syndrome definitions used in the study: (27)

Impetigo: Impetigo is a contagious superficial pyogenic infection of skin; can be bullous or non-bullous.

Ecthyma: Ecthyma is a pyogenic infection of the skin characterized by the formation of adherent crusts, beneath which ulceration occurs.

Staphylococcal folliculitis: superficial folliculitis is an infection of the follicular ostium with *S.aureus*

Furuncle: A furuncle is an acute, usually necrotic infection, infection of hair follicle with *S.aureus*.

Carbuncle: deep infection of a group of contagious follicles with *S.aureus*, accompanied by intense inflammatory changes in the surrounding and underlying connective tissues, including the subcutaneous fat

Sycosis: subacute or chronic pyogenic infection involving the whole depth of the follicle

Definition of Overcrowding used in this study (104)**:**

Room/person ratio wise: Accepted standards for living are one room for two persons, two rooms for three, three for five, four for seven, five or more for 10 persons, additional two for each extra room.

Sex separation wise: Overcrowding is considered to exist if two persons over 9 years of age, not husband and wife of opposite sexes are obliged to sleep in the same room

At the time of registration into the study, a written informed consent was taken from the patient (Annexure 1). All demographic and clinical variables were recorded in a standardized, pilot-tested proforma (Annexure 2). Historical data like recent travel was based on travel to foreign countries over the last 1 year. Data on history of prior antibiotics was noted based on prior available medical documentation or 1 year-recall. Data on previous type of pyoderma was taken depending on prior documentation in previous clinical records, which if not available, was noted as per the description offered by the patient. In the event of incomplete evidence on history or patient being unsure about the details regarding antibiotics, poor response to β-lactam antibiotics or prior pyoderma, the history was recorded as "not reliable". Socio-economic status scoring was done using the modified Kuppuswamy scale, updated in 2007(105) . Data collection in the outpatient was done by the principal investigator alone, with expert advice from the

guide for clinical diagnosis. Size of the largest active lesion was measured only for primary pyodermas. Patient follow up was not a part of the study.

Table 3: Socioeconomic scoring system: (104)

Professsion	Score	Education	Score	Monthly income	Score
Profession or Honors	7	Profession	7	>19575	12
Graduate or post-graduate	6	Semi-profession	6	9788-19574	10
Intermediate or post-high school diploma	5	Clerical, shop owner or famer	5	7323-9787	6
High school certificate	4	Skilled worker	4	4894-7322	4
Middle school certificate	3	Semi-skilled worker	3	2936-4893	3
Primary school certificate	2	Unskilled worker	2	980-2935	2
Illiterate	1	Unemployed	1	<980	1

Socioeconomic status:
26-29: Upper
16-26: Upper middle
11-15: Lower middle
5-10: Upper lower
<5: Lower

In the event that the same patient presented twice during the study period, the data collected at the time of first presentation was considered for the study. The clinical isolates were not differentiated for colonisation or infection, and it was assumed that the isolated organisms were associated with the infection.

Details of clinical examination were entered into the proforma, and pus collection was done under standard sterile methods with sterile cotton-tipped swabs. Pus was collected

following sterile aspiration or incision and drainage, wherever possible. The pus swabs were then transferred to the department of Clinical Microbiology, for diagnostic tests on the same day.

Microbiological assessment to detect the causative organisms was done in the diagnostic laboratory with expert help, under the supervision of the co-investigator.

Culture:

Isolation and identification of *Staphylococcus aureus,* including determination of antibiotic susceptibility was carried out as per the SOP (Standard of protocol) currently followed in Department of Microbiology for the same. The procedure followed is as described below:

On receipt, one swab was used to make a smear for Gram staining whereas the other was inoculated onto blood agar (BA), MacConkey agar (MA) and thioglycollate medium (TG). The BA was incubated at 37^0C in an atmosphere containing 5% CO_2, while the MA and TG were incubated at 37^0C in an ordinary incubator.

After overnight incubation, β-hemolytic colonies which showed gram positive cocci in clusters (GPC) were followed up and were identified to be *S.aureus* if they were tube coagulase and mannitol positive.

Susceptibility to oxacillin and erythromycin was determined by the Kirby-Bauer disc diffusion method as is being performed presently. An isolate was termed as MRSA

(methicillin resistant *Staphylococcus aureus*) if it was resistant to oxacillin and as MSSA (methicillin-susceptible *Staphylococcus aureus*) if it was susceptible to oxacillin.

S.aureus isolates were stored till the end of the study, and were revived later for PCR detection of Panton-Valentine leucocidin.

PCR:

Strains of MSSA and MRSA were subjected to PCR to detect the PVL gene following DNA extraction.

DNA was extracted by boiling, 10^6 CFU/ml (CFU: colony forming units) of either MSSA or MRSA for 10 minutes. The supernatant was used as the template for amplification which was done using primers described by Lina et al(8). The primer sequences are as depicted below:

luk-PV-1: 5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCCA-3';

luk-PV-2: 5'-GCA TCA AST GTA TTG GAT AGC AAA AGC-3'

PCR Mix: The PCR reaction (50 µl) consisted of 25 µl of 2X PCR Master Mix (Fermentas Inc, Glen Burnie, MD, USA), 20 pmol of each primer (Sigma-Aldrich, Bangalore, India) and 5 µl of DNA template.

Size of target: 433 bp

Positive Control: *S.aureus* ATCC 25923

Negative control: *S.aureus* ATCC 43300

PCR amplification and detection: The relevant target was amplified for 30 cycles which included 30 s of denaturation at 94^0 C, 30 s of annealing at 55^0 C, and extension at 72^0 C for 1 minute). The PCR products were resolved by electrophoresis through 1.5% agarose gels (Bangalore Genei, Bangalore, India) containing ethidium bromide. The gel was analysed using a gel documentation system (GelDoc XR, Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis:

Pilot data: We collected preliminary data by evaluating pus samples sent from Dermatology outpatient department as a pilot study over one month (randomly chosen as the month of November 2008). All the patients qualified for community associated infections as per the clinical definition of the 48 hour criterion and there were 76% of staphylococcal isolates of which 27% were MRSA.

Sample size calculation: The sample size was calculated based on the primary objective which was to determine prevalence of MRSA in SSTIs. In the pilot study, the proportion of MRSA among all clinical isolates was 21%. Considering this information and a precision of +/- 5%, the sample size was calculated to be 266. We inflated the sample size by 10% to account for losses such as inadequate sample, etc. Hence, we planned to screen approximately 300 patients.

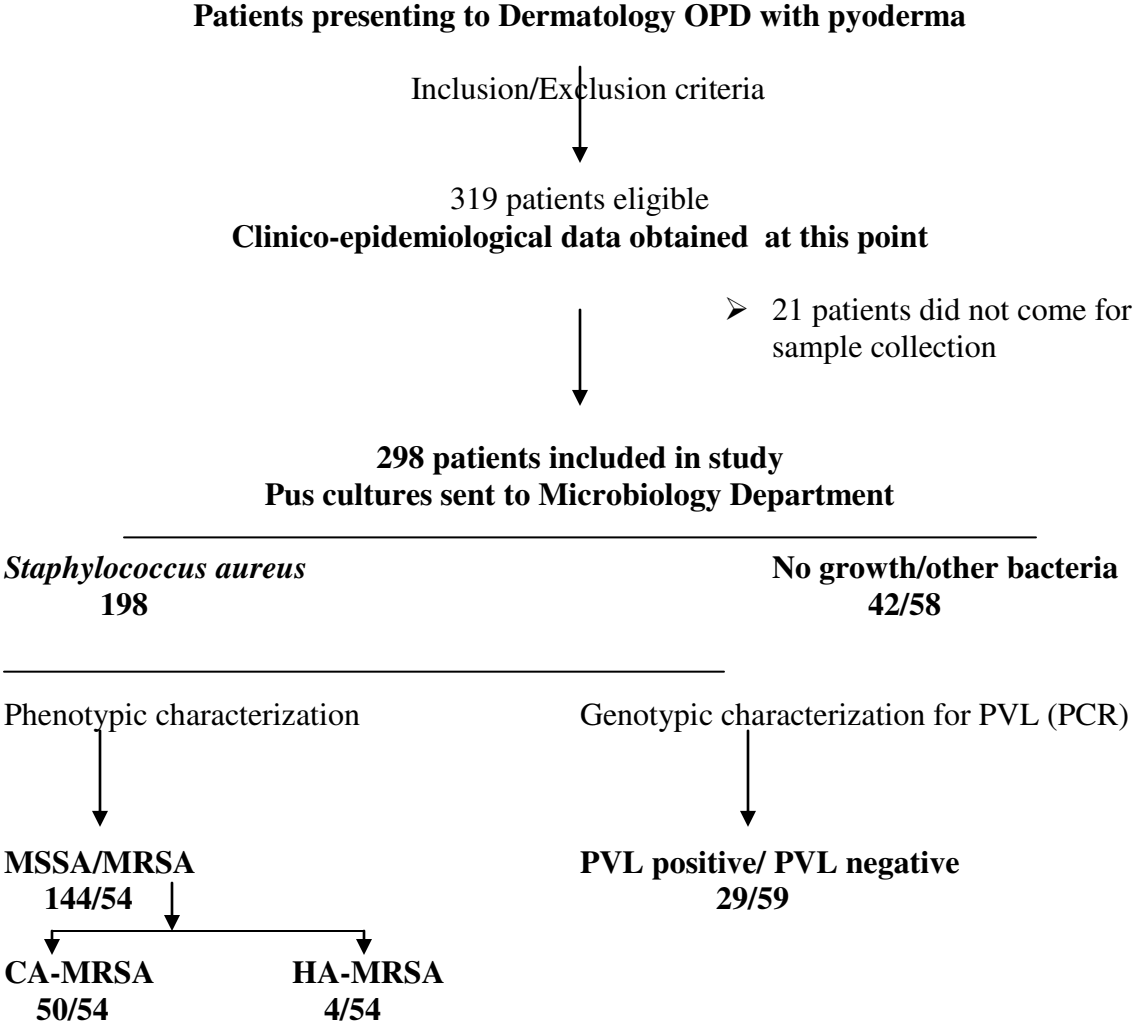
Bias reduction: The assessor in the outpatient was not aware of the outcome, hence interviewer bias was minimised. Recall bias was minimized and adjusted for both MSSA

and MRSA groups by applying the same methodology of data collection for all patients. Clinical diagnosis was corroborated with a senior consultant. Outcome was based on standard methods of culture and sensitivity and standardized and controlled molecular tests like PCR.

Data entry: Data was entered on Epi Info™ Version 3.5.1 software generated data-entry table. All outcome variables for this study were binary variables, denoting the presence or absence of the condition of interest. The baseline socio-demographic variables were either continuous or categorical. Clinical and laboratory information was recorded into a predetermined structured format at the time of data entry.

Data analysis: With the help of STATA/IC Version 10.1 software package, frequency and percentages of all variables were tabulated. Preliminary analysis of demographic and other details was primarily descriptive. The prevalence of MRSA(with 95% confidence interval) was calculated. Comparison of socio-demographic and clinical risk factors between MRSA and MSSA groups was done using two-tailed *t*–test for continuous variables and chi-square test for categorical variables.. For each exposure of interest, prevalence ratio (with 95% confidence interval) was calculated.

RESULTS



Three hundred and nineteen patients met the inclusion criteria during the study period (July 2009 - August 2010) and consented to participate in the study. Of the eligible patients, 21/319 did not come for sample collection although they had consented to participate in the study. Pus culture was taken from 298/319 patients.

Table 3: Bacterial isolates from the pyodermas

Bacteria isolated	N=298	Percentage
No growth	42	14
S.aureus	198	66.4
Beta-hemolytic streptococci	40	13.4
Coagulase negative Staphylococcus	37	12.4
Klebsiella	12	4.03
Enterococcus	11	3.7
Pseudomonas	9	3.0
Proteus	6	2.0
Morganella	3	1.0
Non-fermenting gram negative bacteria	9	3.0
E.coli	10	3.4
Enterobacter	5	1.7
Citrobacter	5	1.7
Hemophilus	1	0.3
Atypical mycobacteria	1	0.3

Of all the samples sent, *Staphylococcus aureus* was isolated from 66.4% (198/298) and from 77.3% (198/256) of all culture positive pyodermas. Among all the *S.aureus* isolates, 72.7% (144/198) were MSSA and 27.3% (54/198) were MRSA. Among all pyodermas, 14% (42/298) did not show growth of any organism.

Single organism isolate was seen in 207 (69.5%) samples, while 40 (13.4%) showed growth of two organisms, 7 (2.3%) showed growth of 3 organisms and 2 (0.7%) had 4 organisms isolated.

Demographic profile of patients:

Table 4: Demographic variables among patients

Variable	Number (N=298)	Percentage
Gender		
Male	184	61.7
Female	114	38.3
Age		
Pediatric	88	29.5
Adult	210	70.5
Geographic distribution		
Vellore district	168	56.3
Rest of Tamilnadu	22	7.3
Others	108	36.4
Occupation		
Pre-school students	46	15.4
School students	45	15.1
College students	32	10.7
Manual labourers	38	12.8
Healthcare workers	27	9.1
Housewives	48	16.1
Self-employed business	19	6.4
Others	43	14.4
Facilities of residence		
Single	45	15.2
Shared	165	55.4
Overcrowding	88	29.4
Socioeconomic status		
Upper	40	13.5
Upper middle	100	33.6
Lower middle	99	33.2
Upper lower	46	15.3
Lower	13	4.4
Medical comorbidities		
Diabetes	24	8.1
Hypertension	18	6.0
Obesity	6	2.0
Hypothyroidism	6	2.0
Immunocompromised	1	0.3
Immunosuppressed	16	5.4
Malignancies	8	2.7

Gender and Age: There were 184 males and 114 females included in the study of whom 29.5% (88/298) were in the pediatric age group (≤ 16 years of age). The median age was 30 years (0-86). (Interquartile range 8 to 46 years)

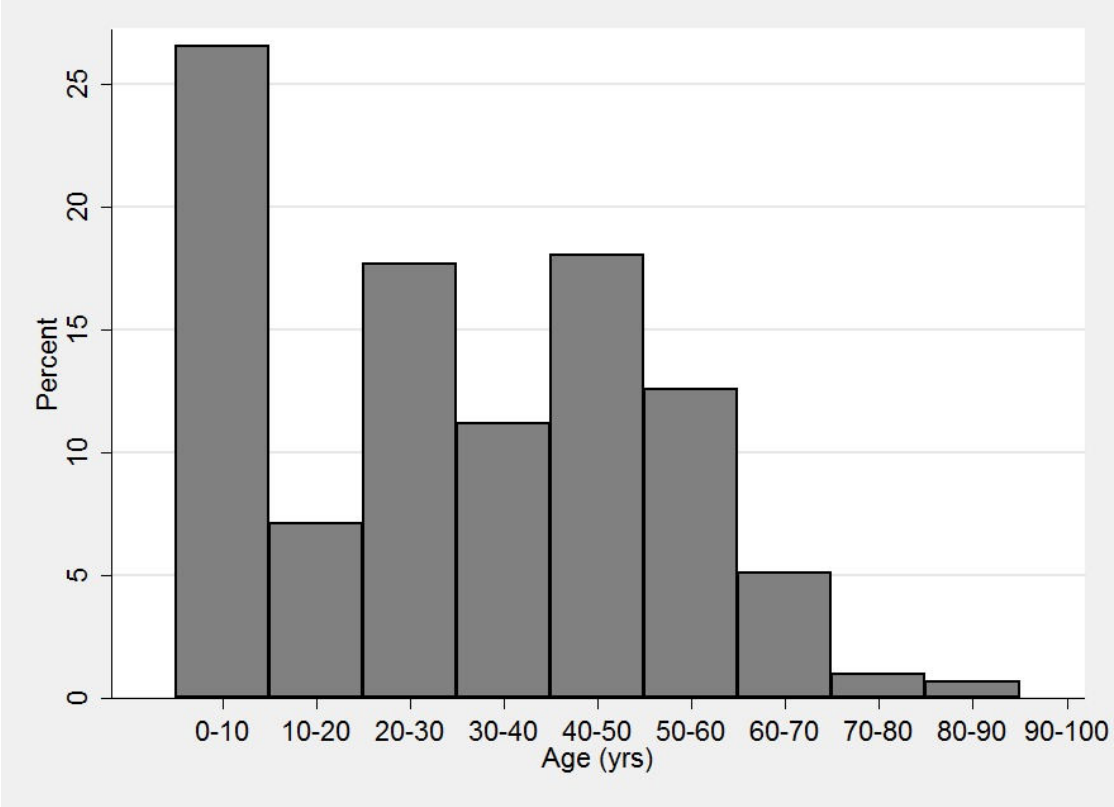


Fig. 3: Age-wise distribution of patients

Geographic distribution of patients: Majority (56.3 %) of patients were from Vellore district, 7.4% belonged to other parts of Tamilnadu and 36.3% were from elsewhere including 3 patients from Bangladesh, 1 each from Bhutan and Belgium and the rest being from other parts of India.

Occupation: Twenty seven (9.1%) patients were health-care workers. The rest of patients comprised of housewives (16.1%), manual labourers (12.8%), self-employed business (6.4%) and other professions.

Facilities of Residence: Forty- five (15.2%) patients lived in single accommodation facility, 55.4% (165/298) in shared accommodation and 29.4% (88/298) in overcrowded places.

Socioeconomic status: Socioeconomic status distribution for the patients studied was as follows: 13.5% (40/298) in the upper status, 33.5% (100/298) in the upper middle and 33.2% (99/298) in lower middle, 15.3% (46/298) in the upper lower and 4.4% (13/298) in the lower status.

Comorbidities:

Medical comorbidities: Medical comorbidities included diabetes 8.1% (24/298), hypertension 6% (18/298), obesity 2% (6/298), hypothyroidism 2% (6/298), immunocompromised 0.3% (1/298) immunosuppressed 5.4% (16/298) and malignancies 2.7% (8/298).

Dermatological comorbidities: Dermatological comorbidities included pemphigus (6), atopic dermatitis (5), psoriasis (4) and one each of Hyper IgE syndrome, Hansen's disease, CHILD syndrome, Nekam's disease and Netherton syndrome.

Prior pyoderma in patients: A total of 45.3% (135/298) patients had history of prior pyodermas, with 25.2% (75/135) having recurrent disease within last 6 months and 44.4% (60/135) with prior episodes occurring more than 6 months apart from the index episode. 114/135 patients had reliable history as per documentation in medical records (79/114) or recall (35/114). Of these 114 patients with prior episodes of pyoderma, the commonest type was furunculosis 58.8% (67/114) followed by impetigo 16.7% (19/114), superficial folliculitis 8.8% (10/114), and abscess 3.5% (4/114).

Prior antibiotic exposure among patients: Patients with exposure to both topical and systemic antibiotics were tabulated as per available medical records. At least single antimicrobial agent exposure (systemic or topical) was noted in 40.9% (122/298) and 22% (66/298) of patients had history of exposure to at least two antimicrobial agents over the past 1 year. Exposure to β-lactam antibiotics was found in 27.5%, to macrolides 9.06%, to quinolones 6.3%, to cotrimoxazole 3.02%, to linezolid 1.34% and to tetracyclines 1.01%. There was history of prior topical Neosporin® use in 6.04%, topical fusidic acid in 3.7% and topical mupirocin in 2.01%.

History of poor response: A total of 8.1% (24/298) patients had history of poor response to β-lactam antibiotics. History of prior MRSA isolation was documented in 6/24 patients.

Clinical Profile:

Table 6: Clinical profile of patients

Variable	N=298	Percentage
Type of pyoderma		
Primary	234	78.5
Secondary	64	21.5
Types of primary pyoderma(N=234)		
Furuncles	115	49.1
Impetigo(non-bullous)	33	14.1
Bullous impetigo	8	3.4
Abscess	32	13.6
Superficial folliculitis	26	11.1
Carbuncles	3	1.3
Others	17	7.3
Types of secondary pyoderma(N=64)		
Eczema	24	37.5
Ulcer	18	28.1
Secondarily infected pemphigus	9	14.1
Others	13	20.3
Symptoms		
Asymptomatic	25	8.4
Pain	268	89.9
Itching	26	8.7
Burning	4	1.3
Others	15	5.1
Signs		
Surrounding erythema	201	67.5
Induration	36	12.1
Lymphadenopathy	25	8.3
Lymphangitis	20	6.7
Necrotic changes	20	6.7
Fever	8	2.8
Site of involvement		
Head and neck	65	21.8
Upper extremities	26	8.7
Lower extremities	83	27.8
Trunk	60	20.4
Axillae	2	0.7
Groins	1	0.3
Palms/ soles	0	0
Disseminated	61	20.7
Size of largest active lesion (primary pyoderma,N=234)		
1-5 mm		
6-10 mm	27	11.5
11-20 mm	85	36.3
21-50 mm	67	28.6
>50 mm	45	19.2
	10	4.3
Number of lesions(primary pyoderma, N=234)		
Solitary		
2-5	45	19.2
6-10	116	49.6
>10	46	19.7
	27	11.5

The majority of patients in our study had primary pyodermas, accounting for 78.5% (234/298), with the rest being secondary pyodermas (21.5%).

Symptoms: At presentation, the most common complaint was pain in 89.9% (268) followed by itching in 8.7% (26), and burning in 1.3% (4). Those who were asymptomatic accounted for 8.4% (25).

The mean duration of symptoms was 6 days (Interquartile range: 5 to 7 days) .

Signs: Erythema surrounding the lesions was noted in 67.5%, induration in 12.1%, locoregional lymphadenopathy in 8.3%, lymphangitis in 6.7%, necrotic changes in 6.7% and fever in 2.8% patients at the time of evaluation.

Clinical types: The primary pyodermas included furuncles and other deep folliculitis in 49.1% (115/234) followed by non-bullous impetigo in 14.1% (33/234), abscess in 13.6% (32/234), superficial folliculitis of Bockhardt in 11.1% (26/234), bullous impetigo in 3.4%(8/234), and carbuncles in 1.3%(3/234). Other primary pyodermas included cellulitis, ecthyma, otitis externa and acute paronychia.

Among secondary pyodermas, secondarily infected eczemas accounted for 37.1%, followed by ulcers (29.03%) and secondarily infected pemphigus (14.5%). Others included secondarily infected psoriasis, post-operative wounds and toxic epidermal necrolysis.

Sites of involvement: The lower extremities was found to be the commonest site for a primary or secondary pyoderma (27.8%), followed by head and neck in 21.8%, disseminated in 20.7%, trunk in 20.4%, upper extremities in 8.7%. The axillae and groins were relatively less common sites to be involved.

Size of largest active lesion: The size of the largest active lesion in primary pyodermas was measured and most of them were found to be 5-10mm in size (36.3%, 85/234), while 1-2 cms size was noted in 28.6% (67/234), 2-5 cms in 19.2% (45/234) and 4.3% (10/234) had lesions larger than 5 cms.

Number of lesions: Lesion count was performed for primary pyodermas. 49.6% of patients had 2-5 lesions, 19.6% had 6-10 lesions, 19% had solitary lesions and 11.6% had more than 10 lesions.

Epidemilogic classification of Methicillin-resistant *Staphylococcus aureus* pyodermas:

Majority of the MRSA pyodermas 92.6% (50/54) were sub-classified as CA-MRSA and the remaining 7.4% (4/50) as HA-MRSA. For the comparative analysis, we considered the differences among all MRSA versus MSSA pyodermas and not as per the sub-classification.

Comparative demographic and clinical profile of patients with MSSA and MRSA

Table 6: Comparative demographic profiles of patients with MSSA and MRSA pyodermas

Variable	MSSA(N=144) n(n/N%)	MRSA(N=54) n(n/N%)	p-value
Gender			
Male	98(74.8)	34(25.2)	0.503
Female	46(69.7)	20(30.3)	
Age			
<5 years	25(65.8)	13(34.1)	0.285
>5 years	119(74.4)	41(25.6)	
Facilities of residence			
Overcrowding	37(57.8)	27(42.2)	0.001
No overcrowding	107(79.9)	27(20.1)	
Socioeconomic status			
Upper	17(80.9)	4(19.1)	
Middle	104(78.2)	29(21.8)	0.002
Lower	23(52.3)	21(47.7)	
Contact sports			
Yes	10(66.7)	5(33.3)	0.584
No	134(73.2)	49(26.8)	
Medical comorbidities			
Yes	25(69.4)	11(30.6)	0.680
No	119(74.1)	43(25.9)	

Demographic factors associated with MRSA infection as compared to MSSA were overcrowding ($p<0.0001$) and low socioeconomic status ($p=0.002$). Genders and age-groups were similar in their association with both MSSA and MRSA.

The occurrence of MRSA in those with history of contact sports was not statistically significant ($p=0.584$).

There was no significant association of MSSA or MRSA with co-morbid medical illnesses ($p=0.680$).

Table 7: Comparative antibiotic exposures among patients with MSSA and MRSA pyodermas

Variable	MSSA(N=144)	MRSA(N=54)	p-value
Systemic antibiotic exposure			
≥2 antibiotics	33(52.4)	30(47.6)	
0-1 antibiotic	111(82.2)	24(17.8)	<0.0001
β-lactam exposure			
Yes	31(51.7)	29(48.3)	<0.0001
No	113(81.9)	25(18.1)	
Macrolide			
Yes	4(20)	16(80)	<0.0001
No	140(78.7)	38(21.3)	
Topical antibiotic exposure			
Yes	13(59.1)	9(40.9)	0.128
No	131(74.4)	45(25.6)	

The association of MRSA with exposure to two or more systemic antibiotics was found to be strongly significant (p<0.0001). Prior exposure to β-lactam antibiotics or macrolides in the past 1 year was significantly associated with MRSA pyodermas (p= <0.0001). Topical antibiotics were not associated similarly with MRSA (p=0.128).

Table 8: Comparative history of prior pyodermas in patients with MSSA and MRSA pyodermas

Variable	MSSA(N=144)	MRSA(N=54)	p-value
History of recurrent pyodermas or household clusters of infection			
Yes	29(50.9)	28(49.1)	<0.0001
No	115(81.6)	26(18.4)	
History of prior pyoderma			
First episode	96(84.2)	18(15.8)	<0.0001
Recurrent episodes	20(64.5)	11(35.5)	
Multiple espidoses	28(52.8)	25(47.2)	

MRSA pyodermas were significantly high in those with history of recurrent pyodermas or household clusters of infection (p=<0.0001).

The prevalence of MRSA was significantly increased among the patients with multiple (35.5%) or recurrent pyodermas (47.2%) as compared to those with first episode (15.8%) of pyoderma (p<0.0001).

Table 9: Comparative clinical profiles of patients with MSSA and MRSA pyodermas

Variables	MSSA (N=144)	MRSA(N=54)	p-value
Type of pyoderma			
Primary	117(70.1)	50(29.9)	0.050
Secondary	27(87.1)	4(12.9)	
Type of primary pyoderma			
Superficial folliculitis	14(100)	0(0.0)	
Furuncles	58(64.4)	32(35.6)	
Carbuncle	0(0.0)	3(100)	
Abscess	12(57.1)	9(42.9)	
Impetigo(non-bullous)	22(84.6)	4(15.4)	
Bullous impetigo	6(100)	0(0.0)	
Clinical features			
Surrounding erythema			
Yes	101(68.2)	47(31.8)	0.015
No	43(86.0)	7(14.0)	
Lympadenopathy			
Yes	11(68.8)	5(31.3)	0.709
No	133(73.1)	49(26.9)	
Lymphangitis			
Yes	7(38.9)	11(61.1)	0.001
No	137(76.1)	43(23.9)	
Induration			
Yes	14(51.9)	13(48.2)	0.009
No	130(76.0)	41(24.0)	
Necrotic changes			
Yes	6(50.0)	6(50.0)	0.068
No	138(74.2)	48(25.8)	
Fever			
Yes	0(0.0)	7(100.0)	<0.0001
No	144(75.4)	47(24.6)	
Site			
Head and neck	29(78.4)	8(21.6)	
Upper extremities	11(55.6)	9(45.4)	
Lower extremities	38(82.6)	8(17.4)	
Trunk	27(69.2)	12(30.8)	
Groins	1(100)	0(0.0)	
Palms/Soles	-	-	
Axillae	1(100)	0(0.0)	
Genitalia	-	-	
Disseminated	37(68.5)	17(31.5)	
Size			
≤ 2 cms	86(71.7)	34(28.3)	0.848
>2 cms	35(74.5)	12(25.5)	
Number of lesions			
Single lesion	14(12.3)	8(17.0)	
2-5 lesions	65(57.0)	25(53.2)	0.129
6-10 lesions	21(18.4)	13(27.7)	
>10 lesions	14(12.3)	1(2.1)	
Panton-Valentine leucocidin			
PVL+	21(72.4)	8(27.6)	0.197
PVL-	35(58.3)	25(41.7)	

Primary pyodermas were significantly associated with MRSA compared to secondary pyodermas (p=0.050).

The prevalence of MRSA in deep pyodermas like furuncles, abscesses and carbuncles was more than those in superficial pyodermas like impetigo or superficial folliculitis. These differences were not statistically relevant because of disproportionate frequencies of individual types of primary pyodermas.

Clinical signs like surrounding erythema (p=0.015), induration (p=0.009), lymphangitis (p=0.001) and fever (p=<0.0001) were significantly associated with MRSA, while necrotic changes (p=0.068) and lymphadenopathy (p=0.709) were not found to be statistically significant.

Lesions over the upper limbs, disseminated disease and trunk had a prevalence of 45.4% 31.5% and 30.8% respectively of isolation of MRSA, while the rest had relatively lower rates of isolation of MRSA.

Size and number of lesions were not significant associations for MRSA pyodermas.

Results of genotypic characterization of *Staphylococcus aureus* for PVL (Table 9) :

PCR for PVL gene analysis was done in 88 retrieved S.aureus isolates. PVL carriage was found 29 in isolates and absent in 59 isolates. Among the PVL positive strains, 27.6%

(8/29) isolates were MRSA and 72.4% (21/29) were MSSA. All the PVL+ MRSA were CA-MRSA strains. There was no significant association between MRSA and PVL carriage, and was found to be present in both MSSA as well as MRSA (p=0.174). None of the HA-MRSA isolates were found to be positive for PVL carriage.

Factors associated with increased risk for PVL associated *S.aureus* pyodermas:

Table 8: Comparative demographic profiles of PVL positive *S.aureus* and PVL negative *S.aureus*:

Variables	PVL negative (N=59)	PVL positive (N=29)	p-value
Occupation			
Pre-school children	16(76.1)	5(23.9)	
School children	9(56.2)	7(43.8)	
College students	4(44.4)	5(55.6)	
Manual labourers	5(62.5)	3(37.5)	
Health-care workers	3(42.9)	4(57.2)	
Others	22(81.5)	5(18.5)	
Facilities of residence			
Overcrowding	19(61.3)	12(38.7)	0.429
No overcrowding	40(70.2)	17(29.8)	
SES			
Upper	4(57.1)	3(42.9)	0.622
Middle	41(70.7)	17(29.3)	
Lower	14(60.9)	9(39.1)	

Although 17/29 PVL positive *S.aureus* were isolated from children and young adults (age<30 years), there was no statistically significant difference among the various age groups (p=0.477).

Table 9: Comparative clinical profiles of PVL positive *S.aureus* and PVL negative *S.aureus*

Variables	PVL negative (N=59)	PVL positive (N=29)	p-value
Type of pyodermas			
Primary	54(66.7)	27(33.3)	0.780
Secondary	5(71.4)	2(28.6)	
Type of primary pyodermas			
Superficial(others)	17(77.3)	5(22.7)	0.123
Deeper(furuncles/carbuncles/abscess)	32(57.1)	24(42.9)	
Clinical features			
Necrotic changes			
Yes	1(12.5)	7(87.5)	0.001
No	58(72.5)	22(27.5	
Induration			
Yes	7(50.0)	7(50.0)	0.139
No	52(70.3)	22(29.7)	
Lymphangitis			
Yes	7(53.9)	6(46.1)	0.273
No	52(69.3)	23(30.7)	
Surrounding erythema			
Yes	47(64.4)	26(35.6)	0.241
No	12(80.0)	3(20)	
Size			
<2 cms	42(72.4)	16(27.6)	0.136
>2 cms	17(56.7)	13(43.3)	
Site			
Head and neck	12(75.0)	4(25.0)	
Upper extremities	3(37.5)	5(62.5)	
Lower extremities	14(77.8)	4(22.2)	
Trunk	10(66.7)	5(33.3)	
Groins	1(100)	0(0.0)	
Palms/Soles	-	-	
Axillae	1(100)	0(0.0)	
Genitalia	-	-	
Disseminated	18(62.1)	11(37.9)	

The clinical features were tested for PVL association and only necrotic changes were found to be a significant association (p=0.001).

Pyodermas like furuncles, carbuncles or abscesses were noted to have PVL positive *S.aureus* in 42.9%; however the association was not statistically significant (p=0.123).

DISCUSSION

Pyodermas constitute a significant burden of cutaneous diseases across the world, and more significantly, in the tropical countries.(102) Majority of these are caused by Staphylococcus aureus. In contrast with high-income countries, *S.aureus* disease ranks low on the public-health agenda in developing and underdeveloped countries. However, neglected status as a developing world pathogen does not equate with low rates of disease.(106) The prevalence of methicillin-resistant *S.aureus* (MRSA) infection across much of resource-limited Asia including India is largely unknown. There are few prospective studies from India on the prevalence of MRSA in community-associated pyodermas, the presence of virulence factors like Panton-Valentine leucocidin and risk factors associated with the same.(12)(49) The prevalence of MRSA has been as variable as less than 1% to 9.6% from dermatology outpatient based studies from India(12)(49), which contradicts with pilot data in our institution revealing 27% *S.aureus* to be methicllin-resistant in pyodermas. These lacunae and discrepancies in existing data prompted us to undertake a prospective cross-sectional study to address these key issues.

The prevalence of *S.aureus* was found to be maximum among all bacteriae isolated from SSTIs (77.3%) in our study as was described in other studies as well. (20)(25) Of the *S.aureus* isolates, 27.3% (54/198) were found to be methicillin-resistant. CA-MRSA accounted for 92.6% (50/54) and HA-MRSA for 7.4% (4/54) of the MRSA strains isolated. Del Guidice et al.(5) demonstrated 11% (22/197) of MRSA from 197 isolates of *S.aureus* from primary and secondary pyodermas. Of the 22 MRSA isolates in the same

study, 6 were classified as CA-MRSA,15 as HA-MRSA and 1 as MRSA strain of unknown origin. Jappe et al.(4) demonstrated 52.4% (130/248) of all pyodermas presenting to a tertiary care university hospital in Heidelberg, Germany, to be associated with *S.aureus*, with 7.3%(18/130) of all *S.aureus* isolates being methicillin-resistant. Out of 18 isolates of MRSA in the Heidelberg study, 4 were sub-classified as CA-MRSA.

For our study, we limited ourselves to the definitions of CA-MRSA and HA-MRSA, provided by Salgado et al.(36), which is the same as that adopted for clinicians(CDC,2005). (52) By this criteria the majority of patients were CA-MRSA(50/54, 92.6%). Our study included 9.2% of healthcare-workers, who could possibly be circulating HA-MRSA strains, but were not included under the HA-MRSA group as per the criteria used. This criteria was also used in outpatient studies by Del Guidice(5) and Jappe et al.(4) On the other hand, prior Indian studies have used the 48 hour post-admission nosocomial isolates of MRSA as HA-MRSA, thereby assuming all outpatient cases to be community-associated; hence the prevalence data are not comparable due to use of different set of criteria. Conversely, CA-MRSA can be acquired in the hospital as well, due to increasing prevalence of CA-MRSA. Given the complex epidemiology of CA-MRSA strains in health care settings and the circulation of HA-MRSA strains that occurs in the community, establishing a clear delineation between CA-MRSA and HA-MRSA strains has not been possible. CDC investigators have used a third category of MRSA infections, "health care-associated, community-onset" MRSA (HACO-MRSA) infection (107); this category includes cases that would be HA-MRSA infections by history of health care exposure but have onset in the community. This tripartite classification scheme, HA-, CA-, and HACO-MRSA, still has limitations

because a history of exposure to a health care setting does not exclude the possibility of MRSA acquisition and infection in the community.

It was interesting to note that more than 30% (85/298) of the total study population in our study included children below 16 years. In a pediatric cohort of pyodermas studied by Sardana et al.(48) in New Delhi, the prevalence of MRSA was only 6.9%, while our study showed a prevalence of 35.2% (19/54).

There was no occupation showing an increased association with MRSA. Other occupations that are known to have high prevalence of MRSA pyodermas like military recruits, professional athletes, veterinarians or meat handlers (15)(22) were not encountered in our study. MRSA infection was not significantly associated among health-workers in our study. In a study by Reich-Schupke et al.(103) conducted at a dermatology department in Germany, both patients and employees of the department were prospectively enrolled to study nasal carriage of MRSA The nasal carriage between hospital employees and outpatients was not significant(108). Although the presence of PVL in HA-MRSA is characteristically not seen (4)(107), nosocomial transmission of PVL-carrying MRSA leading to fatality in a healthcare worker has been reported.(109) This could imply CA-MRSA strains being spread to health-care workers in the hospital.

The demographic factors significantly associated with MRSA in our study were overcrowding and low SES. Our study showed 29.4% (87/298) of the study population living in overcrowded spaces. These patients were more susceptible to developing

pyodermas as a result of poor hygiene, fomite spread and direct spread in household clusters of pyodermas in overcrowded environments. (87)(110) Although overcrowding has particularly not been described in earlier studies to be an epidemiological association of MRSA, there is indirect evidence of the same. Pallin et al(87) have suggested that the higher isolation of MRSA from EDs in USA could be possibly because of patients presenting from low SES and staying in overcrowded places.(110) Underserved urban communities have been found to be risk factor for MRSA carriage in the US or developing countries like Nigeria.(22)(102) There are no other studies that have assessed socioeconomic status by a scoring system to determine prevalence of CA-MRSA.

A prior retrospective study at our center in 1996 by Pulimood et al. showed 24% of all *S.aureus* isolates from blood or pus to be methicillin-resistant.(40) However, the study did not however elaborate on prevalence of MRSA in SSTIs or the proportion of nosocomial isolates separately. Studies from other centres in India have shown variable prevalence rates of 11.8% in Mangalore(11), 9.6% in New Delhi(49) and 0.9% in Mumbai.(12) In our sudy, the overall prevalence of MRSA in any pyoderma was 18.1%, while MRSA among all *S.aureus* isolates was 27.3%. These figures were higher than other available reports from India.

Bae et al(9) demonstrated high prevalence of PVL positive *S.aureus* across the world. Goering et al (21) showed PVL positive stains (2 PVL+ MRSA, 15 PVL+ MSSA) from India among other centers in a multicentric study. A cohort of miscellaneous CA-MRSA and HA-MRSA(111) PVL positive strains were reported by Nagarajan et al(66) from

Chennai. In a study by Nadig at al (67) from Bangalore, nasal swabs from patients with SSTIs, brain abscesses and meningitis showed PVL positive *S.aureus* isolates. PVL positive isolates have been reported in genotyped S.aureus from Mumbai by D'Souza et al.(111) PVL positive MRSA has been isolated in an adolescent from Chennai with furuncles, sepsis and pneumonia.(17) PVL positive *S.aureus* patients in our study were isolated from patients belonging to the states of Tamilnadu, Karnataka, Andhra Pradesh, West Bengal, Jharkhand, Tripura from India and a single patient from Bhutan. This reveals the emergence of PVL positive *S.aureus* in the Indian subcontinent. Further characterization of strains is needed to determine if they are similar to other clones from rest of the world.

Although diabetes, hypertension, dyslipidemia, obesity were noted in a few patients, there was no statistically significant association seen. Diabetes(4)(112), obesity(103) and chronic leg ulcers(4)(108) have been described for increased carriage of MRSA and PVL. Due to the small numbers of *S.aureus* isolates from immunosuppressed patients, an association with MRSA could not be ascertained. 2 patients with atopic dermatitis were found to have MRSA infection. Lo et al. (59) demonstrated increased carriage of MRSA in atopic dermatitis in a cohort of Taiwanese children.

Various studies have shown prior exposure to systemic antibiotics to be associated with acquisition of MRSA. (4)(15)(94)(113) This was also seen in our study.

The association of recurrent pyodermas or household clusters of infection was found to be significantly associated with MRSA isolation. This risk factor has also been described widely in prior studies. (16)(22) (24)(36)(80)(110)

There was a statistically significant difference of MRSA being more prevalent in the primary pyoderma group. Most prospective studies have not evaluated this difference in MRSA epidemiology. MRSA has been described in primary infections like cutaneous abscesses, furuncles(5)(8) as well as diabetic foot ulcers(112), atopic dermatitis(61) and chronic venous leg ulcers.(4)(108) Among secondary pyodermas, we did not differentiate between infection and colonization as mentioned in the methodology.

Cutaneous infections like furuncles, abscesses and carbuncles were associated with higher rates of MRSA isolation as compared to other pyodermas. Complicated pyodermas like pyomyositis, necrotizing fasciititis were not encountered in the study. There were few cases of felon/acute paronychia for statistical comment.

There was no statistically significant association between CA-MRSA and PVL, as PVL was found commonly among MSSA(36.4%) as well as MRSA(24.2%) in our study. MSSA actually had nearly 14% increased prevalence of PVL among the isolates subjected to PCR analysis. None of the isolates sub-classified as HA-MRSA were positive for PVL.

Contrary to earlier studies(5)(8) that have suggested PVL to be an epidemiological marker of CA-MRSA, our study shows PVL to be a marker of community associated

strains of *S.aureus*, irrespective of their methicillin-resistance status. PVL positive MSSA has been particularly noted to be of higher prevalence in South Africa and India by Goering et al. (21) Hence, it is possible that in India, the prevalence of PVL in MSSA is higher than described in other European studies.

The only significant association of PVL in this study was necrotic changes associated with staphylococcal pyodermas. Dermatonecrotic property of PVL has been described in several reports and studies (22)(70) because of which it has been confused with spider-bites. Jappe et al.(4) noted younger patients and deep skin infections to be associated with PVL infections. Our study did not show any such statistically significant correlation although 17/29 PVL isolates were seen among children and young adults below 30 years of age.

Higher prevalence of MRSA and PVL individually in primary infections like furunculosis and abscesses is in concurrence with other studies. (5)(8) PVL is also not necessarily and universally associated with all deep infections like abscesses or furuncles and contradicts earlier studies.(7)(8) This is supported by more recent studies of similar designs(4)(56)(57) that PVL is not necessarily a part of all deep infections. MRSA may be associated significantly with more severe infections but still are clinically indistinguishable (95) as MSSA can also manifest with similar manifestations.

This prospective study on pyodermas had a larger sample size than prior studies of similar interest (4)(5) and design done elsewhere in the world. There is no prior published data from India on the associations of MRSA and PVL carriage. In summary, *S.aureus* is

the most common implicated organism in SSTIs with a prevalence of 66.4%. MRSA prevalence (27.3%)in our study was higher compared to other published Indian studies. (11)(12)(49) Demographic risk factors in Indian subjects for MRSA are similar to those in earlier described studies like overcrowding, low socio-economic status, recurrent infections or household clusters of infection and prior systemic antibiotic use like β-lactams and macrolides.

Our study demonstrated association of low SES with MRSA infections for the first time, using a standard scoring system. We also note that PVL did not correlate with methicillin-resistance; however larger studies are needed from India to correlate between PVL and *S.aureus* pyodermas, its association affecting antimicrobial susceptibility and association with outcome of disease.

CONCLUSIONS

- Staphylococcus aureus was the most common organism (66.4%) isolated from primary or secondary pyodermas.
- The prevalence of MRSA among all pyodermas was 18.1% and among all *S.aureus* pyodermas was 27.3%. This prevalence was higher compared to prior studies done from India in the past(41)(48)(50) or in the contemporary period(11)(49). CA-MRSA was noted in 92.6% of all MRSA pyodermas.
- Significant associations for increased risk of MRSA pyodermas in this study were overcrowding, low SES, recurrent pyodermas or household clusters of infection and prior exposure to systemic antibiotics like β-lactams and macrolides.
- Primary pyodermas are more often associated with MRSA than secondary pyodermas. Primary pyodermas with surrounding skin erythema, induration, lymphangitis and fever were significantly associated with MRSA.
- Panton-Valentine leucocidin carrying S. aureus are prevalent in India, across different geographical areas. They were however, not significantly associated with MRSA and were seen more commonly among MSSA strains. PVL expression was not seen among isolates with hospital-associated risk factors.
- PVL, a dermatonecrotic toxin was significantly associated with necrotic skin changes.
- Our data suggests continued surveillance of MRSA among dermatology outpatients as dermatologists are first in line to note the changing epidemiology of *S.aureus.*

Limitations

- Presence of PVL in all isolates of *S.aureus* would allow a better estimate of its association with SSTIs.

- Follow up among these patients was not part of this cross-sectional study; hence a correlation of management and outcome could not be studied.

- Anti-microbial susceptibility besides for oxacillin and erythromycin was not uniformly studied among the *S.aureus* isolates.

Future directions

- Antimicrobial studies on PVL positive and negative strains will help in determining if there is any significant difference in their susceptibility profiles.

- The emergence of PVL among *S.aureus* in the Indian subcontinent is now established. Further studies can be done to look for clonality of these strains in comparison to those isolated in other parts of Asia or Europe.

SUMMARY

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA), a known nosocomial pathogen, has been increasingly reported globally in patients from the community, without hospital-related risk factors. Pyodermas represent majority of the disease burden caused by MRSA. Community associated-MRSA (CA-MRSA) and methicillin-susceptible *S.aureus* (MSSA) may harbour Panton-Valentine leucocidin (PVL) which is associated with necrotic and severe infections. There is no published data on demographic and clinical features of MRSA and PVL associated pyodermas from India.

Objective: To study the clinical and microbiological features of *S.aureus* pyodermas, and evaluate association of demographic and clinical factors with MRSA and PVL.

Methodology: An open, prospective, cross-sectional study was carried out in the Department of Dermatology, Venereology and Leprosy, Christian Medical College and Hospital, Vellore from July 2009 to July 2010 for patients presenting with pyodermas. Pus cultures were obtained by standard methods and *S.aureus* isolates were further characterized for MSSA and MRSA phenotypically and genotypic characterization for presence of PVL was done by PCR.

Results: 298 patients (184 males, 114 females) were enrolled into the study. *S.aureus* was the most commonly isolated organism 66.4% (198/298). Among all *S.aureus*, 27.3% (54/198) were MRSA. CA-MRSA accounted for 92.6% (50/54) of MRSA, as per CDC

classification criteria for clinicians. 29.5% (88/298) of patients were in the pediatric age-group. The majority of patients had primary pyodermas 78.5% (234/298), with the rest being secondary pyodermas (21.5%). Furuncles (49.1%) and non-bullous impetigo (14.1%) were the most common presentations among primary pyodermas. Demographic factors associated with MRSA infection as compared to MSSA were overcrowding ($p<0.0001$) and low socioeconomic status ($p=0.002$). The association of MRSA with exposure to two or more systemic antibiotics ($p<0.0001$), prior exposure to β-lactam antibiotics or macrolides in the past 1 year ($p= <0.0001$) and those with history of recurrent pyodermas or household clusters of infection ($p=<0.0001$) was significant. Primary pyodermas were significantly associated with MRSA compared to secondary pyodermas ($p=0.050$). The prevalence of MRSA in deep pyodermas like furuncles, abscesses and carbuncles was more than those in superficial pyodermas like impetigo or superficial folliculitis. Clinical signs like surrounding erythema ($p=0.015$), induration ($p=0.009$), lymphangitis ($p=0.001$) and fever ($p=<0.0001$) were significantly associated with MRSA. There was no significant association between MRSA and presence of PVL ($p=0.174$). None of the HA-MRSA isolates were found to be positive for PVL carriage. Necrotic changes in primary pyodermas were found to be significantly associated with PVL ($p=0.001$).

Conclusion: The prevalence of MRSA in this study was higher compared to other studies from India. PVL positive *S.aureus* are prevalent in India and were isolated in both MSSA and MRSA. Further studies are required on larger populations from India to study association of PVL with antimicrobial susceptibility and outcome of disease.

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Annexure I.

Informed Consent form to participate in a research study

Study Title: To study the clinico-microbiological profile of Staphylococcus aureus in Dermatology outpatients

Study Number:
Subject's Initials: _____ Subject's Name: _____
Date of Birth / Age:_____
Please initial box
(Subject)
(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]
(ii) I understand that my participation in the study is voluntary and that I am
free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]
(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]
(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]
(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:_____
Date: _____/_____/_____
Signatory's Name: _____

Signature of the Investigator: _____
Date: _____/_____/_____
Study Investigator's Name: _____

Signature of the Witness: _____
Date:_____/_____/_____
Name of the Witness: _____

STUDY INFORMATION SHEET

1) **Explanation of the purpose of the research**

A research study is a special way to find out about something. It is a common to see patients coming to Dermatology with pus or boils on the skin. The medicines that are used may not work at times since the bug causing the infection may be resistant to it. The bug's resistance can be known by a culture and sensitivity test which takes about three days. I intend to find predictors that will help doctors decide when the skin infection might be caused by a resistant bug called MRSA

This study also involves finding a certain factor in these bugs called PVL which is known to increase the severity of the infection. There is no information in India whether this factor exists or not in the bugs. If this factor is common and study shows significant problems because of it, this could be offered as a diagnostic test in the hospital. Knowledge of these factors and resistance patterns will help us treat patients better.

2) **Expected duration of the subject's participation:**

We will see you only once in the study period

3) **Procedures to be followed, including invasive procedures:**

The doctor will do detailed clinical examination and note down the data in a special proforma. We will collect pus with swabs for further tests, which is part of your regular care. We may perform incision and drainage (involves a small cut on skin) or puncture the pus collection with a needle and aspirate pus.

4) **Any reasonably foreseeable risks or discomforts to the subjects**

We want to tell you about somethings that might hurt or upset you if you are in this study.

The most common side effects from incision and drainage/sterile aspiration include:

- Pain
- Bleeding

5) **Any benefits to the subject.**

Any method to drain the pus has therapeutic benefit and this procedure is also done for diagnosing which bug is causing your problem, and which all drugs can be used to treat the infection.

6) **Disclosure of specific appropriate alternative procedures or therapies:**

Not applicable

7) **Confidentiality of records**

Every reasonable effort will be made to keep your records confidential, However, while you are in this study we do have to let some people look at your records. The IRB (for the protection of human subjects in research), other regulatory agencies responsible for overseeing research (if applicable) and the co-investigators. We will keep your records confidential unless we are required by law to share any information.

8) **Trial treatment schedules:** not applicable

9) **Compensation and /or treatment(s) available to the subject in the event of a trial related injury:**

Not applicable

10) **Nature of participation in the study:**

It is not compulsory for you to participate in the study. It's up to you. If you even decide to withdraw from the study later at any point of time you are eligible to do so. All that you have to do is to tell us. Your refusal to participate in the study will not involve any penalty or would not affect your treatment in any aspect, you can continue your treatment here as before.

Annexure 2. Proforma for Clinico-microbiological profile of *Staphylococcus aureus* in Dermatology outpatients

Socio-demographic data

Patient name: **Hospital No:** **Study ID no:**
Age: Neonate/Infant/1- 2 yrs/2-4 yrs/5-15 yrs/16-60 years/> 60 years
Occupation: Pre-school/Students(school)/Students(college students or higher education)/professional atheletes /military personnel/manual labourer/others
Place of stay: In Vellore/ Rest of Tamil Nadu/Others
Facilities of residence: Single room/shared room with one/shared with more than one/overcrowding
History of contact sports: **High risk behaviour:** Y/N **Sexual orientation:**
History of hospitalization, receipt of hemodialysis, or residence in a long-term care facility during the previous year; surgery during the previous one year ; the presence of an indwelling catheter or a percutaneous device at the time the culture sample was obtained; or previous isolation of MRSA:
History of medical comorbidities:
diabetes/hypertension/hypothyroidism/dyslipidemia/obesity/immunocompromised/on immunosuppresants/others(_____)
History of recurrent(twice or more in six months) primary pyodermas or household clusters of infection: yes/no/ history not reliable
History of poor response to beta-lactam antibiotics: yes/no/ history not reliable
History of exposure to two or more groups of antibiotics in the past one year:
yes/no/history not reliable
If yes, what antibiotics: beta-lactams/quinolones/macrolides/_____
Topical antibiotics_____
History of recent travel: yes/no **If yes, to:**
Education status of family member(highest in the family):
Professional or honors(7)/graduate or postgraduate(6)/intermediate or post-high school diploma(5)/high school certificate(4)/middle school certificate(3)/primary school certificate or literate(2)/illiterate(1)
Occupation status:
profession(10)/semi-profession(6)/clerical,shop owner,farmer(5)/skilled worker(4)/semi-skilled worker(3)/unskilled worker(2)/unemployed(1)
Family income per month:
(in Rs. Per month) >/= 19575(12)/19574-9788(10)/9787-7323(6)7322-4894(4)/4893-2936(3)/2935-980(2)/<980(1)
Score:
Socioeconomic status:
Upper(26-29)/ Upper middle(16-25)/Lower middle(11-15)/Upper lower(5-10)/Lower(<5)

Clinical proforma

Duration of Symptoms:
History of pyoderma-First episode/Multiple episodes but six months apart/recurrent episodes:
If recurrent or multiple: what was the type of pyoderma earlier:
History of symptoms: pain/ burn/itch/asymptomatic/others

Clinical syndrome:

Primary pyoderma:

Morphological type of lesion	Site/s	Size	Number	Surrounding erythema	Induration	Necrotic changes	Lymphangitis	Lymphadeno pathy	Fever

Secondary pyoderma:
Primary diagnosis:

Additional Information:

Microbiological data:

Bacteria isolated:_____/_____/_____/_____/_____
If *S.aureus:*

MSSA/MRSA:
PVL: positive/negative

hospno	pvl	name	age	agecategor	occupation	otheroccup	placeofstay	facilitiesfr	contactspo	highriskbeh	sexualorien	medicalcon	hamrsariskl	othercomo	historyofre	historyofpo	historyofpo	antibiotice
000757c	0	Indrakala	29	6	8	housewife	1	3	0	0	0	7	1	0	0	0	0	0
003803D	1	Jayashanke	42	6	7	0	2	1	0	0	0	1	0	0	0	0	0	0
006116D	0	Hamshavar	52	6	8	housewife	1	1	0	0	0	0	1	0	0	1	0	0
011298B	0	Hemalatha	50	6	8	housewife	1	3	0	0	0	1	0	0	0	0	0	0
014284B	0	bALKISH	28	6	3	0	1	4	0	0	0	7	0	CBDC		0	0	0
025797B	1	pRABHAKA	38	6	7	0	1	2	0	0	0	0	0	0	0	0	0	0
029608D	0	sHEELA	23	6	3	0	2	1	0	0	0	0	0	0	0	0	0	0
038070C	0	Udaishanke	50	6	8	engineer	4	2	0	0	0	0	0	0	0	0	0	1
040699D	0	Vaigai's bat	3	4	1	0	1	4	0	0	0	0	0	0	0	1	0	0
052092D	0	Nitya	3	4	2	0	1	1	1	1	0	0	0	0	0	0	0	0
057325d	0	srinivasulu	73	7	8	retired teac	4	1	0	0	0	1	0	0	0	0	0	0
058207D	0	Rajesh Mar	20	6	3	0	1	2	1	0	0	0	0	0	0	1	0	0
058522D	0	Sophia	34	6	7	0	1	3	0	0	0	0	0	0	0	0	0	0
061554B	0	Vellai	60	7	6	0	1	3	0	0	0	1	0	0	0	0	0	0
062851D	0	Prarthana	20	6	3	0	1	3	0	0	0	0	0	0	1	1	0	1
092089d	0	Sathiya's ba	1	3	1	0	1	3	1	0	0	0	0	0	1	0	0	0
092175D	0	Usha Rani's	2	3	1	0	1	4	0	0	0	0	1	0	0	0	0	0
115123D	0	Latha's bab	2	3	1	0	1	4	0	0	0	0	0	0	0	0	0	0
130677D	0	Latheef	38	6	6	0	1	4	0	0	0	1	0	0	1	0	0	1
130970C	0	Dhanusurya	8	5	2	0	1	3	1	0	0	0	0	0	0	0	0	0
133819D	0	Swapan Kur	58	6	8	clerk	4	1	0	0	0	0	0	0	0	0	0	0
134261A	0	RUCKMANI	55	6	8	housewife	1	4	0	0	0	1	0	0	0	0	0	0
135923D	0	Sathiya's ba	2	3	1	0	1	4	0	0	0	0	0	0	0	0	0	0
136111D	0	Shanmugap	25	6	7	0	1	2	0	0	0	0	0	0	0	0	0	0
137501D	0	Merlin	2	3	1	0	1	3	0	0	0	0	1	0	0	0	0	1
158097C	0	Jim Evans	25	6	3	0	3	1	0	0	0	5	1	Hyper IgE		1	1	0	1
162064D	0	Parimals's b	1	2	1	0	4	4	0	0	0	0	0	0	1	0	0	0
167203D	0	Gopinathar	29	6	8	clerk	1	3	0	0	0	0	6	0	1	0	0	1
172308D	1	Vijaylakshm	1	2	1	0	1	1	0	0	0	0	6	0	1	0	0	0
183171A	0	Mani	55	6	7	0	1	4	0	0	0	1	0	0	0	0	0	0
222296B	0	Maragatha	46	6	8	0	1	2	0	0	0	0	0	0	1	0	0	0
230816A	0	Manoharan	55	6	7	0	1	2	0	0	0	0	0	0	0	0	0	0
231846C	0	Pushpa's ba	7	5	2	0	2	4	1	0	0	0	0	0	1	1	0	1
238006B	0	Sadrack	2	3	1	0	1	3	0	0	0	0	6	0	0	0	0	0
238006b	0	Sadrack	12	5	2	0	1	3	1	0	0	0	0	0	0	0	0	0
240367D	1	Sarma	68	7	8	retired emp	4	2	0	0	0	1	0	0	0	0	0	0
247262C	0	Sweety	7	5	2	0	1	1	0	0	0	0	0	0	0	0	0	0
253549D	0	Kishori Lal	43	6	6	0	4	4	0	0	0	0	0	0	0	0	0	0
253868D	0	Amidhi's ba	1	2	1	0	1	1	0	0	0	0	0	0	1	0	0	0
258591D	0	Anant	44	6	8	grocer	4	2	0	0	0	0	0	0	0	0	0	0
261378A	0	Babu	27	6	7	0	1	3	0	0	0	0	0	0	1	0	0	0
267591B	0	Violet	57	6	0	housewife	2	3	0	0	0	1	0	0	0	0	0	1
268147D	0	Ebenezer	20	6	3	0	1	3	0	0	0	0	0	0	1	0	0	0
281981	0	Natarajan	46	6	8	0	1	2	0	0	0	0	0	0	0	0	0	0
282166D	0	Gomathi	22	6	7	0	1	3	0	0	0	0	0	0	0	0	0	0
295152D	0	Sibi Raj	1	2	1	0	1	4	0	0	0	0	0	0	0	0	0	0
299434D	0	Francis	52	6	8	clerk	4	1	0	0	0	7	1	Adenocarci		0	0	0	0
300486D	0	Elongovan	55	6	8	lawyer	1	2	0	0	0	7	1	pemphigus		1	0	0	1
309232	0	Lalitha	59	6	8	housewife	1	2	0	0	0	6	1	0	0	0	0	0	0
317719B	0	Ramraj	22	6	7	0	1	2	0	0	0	0	0	0	0	0	0	0	0
319980C	0	Selvaraj	52	6	6	0	1	3	0	0	0	0	0	0	0	0	0	1	0
320966C	0	Elizabeth V	25	6	7	0	1	3	0	0	0	0	0	0	0	0	0	0	0
325767D	0	Vikash	17	6	2	0	4	1	0	0	0	0	1	tuberculosi		0	0	0	1
326618D	0	Lakshmi	42	6	8	housewife	1	1	0	0	0	0	0	0	1	0	0	1	0
329238D	0	Illayaraja	26	6	7	0	1	2	0	0	0	0	0	0	0	0	0	0	0
332235D	1	Nilesh	20	6	3	0	4	2	0	0	0	0	0	0	0	0	0	0	0
340764D	0	Vikram	28	6	6	0	1	4	0	0	0	0	0	0	0	0	0	0	0
345682C	1	Dilip	7	5	2	0	4	3	0	0	0	0	0	0	0	0	0	0	0
347420D	0	Rajeshwari'	1	1	1	0	1	3	0	0	0	0	0	0	0	0	0	0	0
347440D	0	Kevin	1	2	1	0	1	3	0	0	0	0	0	0	0	0	0	0	0
347440d	0	Ammukutty	1	3	1	0	1	3	0	0	0	0	0	0	0	0	0	0	0
35682c	0	Dileep	6	5	2	0	1	3	1	0	0	0	0	0	1	1	0	1	0
357124D	0	Raghupathy	36	6	6	0	1	4	0	0	0	0	0	0	0	0	0	0	0
364950D	0	Kanchan	44	6	1	0	4	2	0	0	0	0	0	0	0	0	0	0	0
367981D	0	Sumit	54	6	8	business	4	3	0	0	0	0	0	0	0	0	0	0	0
369066D	1	Karma Tenz	15	5	2	0	4	4	0	0	0	7	1	ALL		0	0	0	0
369086D	0	Punitha	31	6	8	housewife	1	4	0	0	0	1	0	0	1	1	0	1	0
371365D	1	Yogeshwara	1	2	1	0	2	4	0	0	0	0	0	0	1	0	0	0	0
372957D	0	Sultana's ba	1	1	1	0	1	4	0	0	0	0	0	0	0	0	0	0	0
376013D	0	Bijaya	45	6	8	housewife	4	2	0	0	0	7	1	CML		0	0	0	1
379688C	0	Bhuvanesh	6	5	2	0	1	4	1	0	0	0	0	0	1	0	0	0	1
380182A	0	Jeyaraman	59	6	6	0	1	2	0	0	0	0	0	0	1	1	0	1	0
385789D	0	Menaga's b	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
386223C	0	Manopriya	30	6	7	0	1	2	0	0	0	5	0	0	0	0	0	0	0
389271D	1	Abdul khad	1	1	1	0	1	4	0	0	0	0	0	0	0	0	0	0	1
392327D	0	Debasish	5	5	2	0	1	3	1	0	0	0	0	0	0	0	0	0	0
394666B	0	Balaji	30	6	6	0	1	2	0	0	0	0	0	0	0	0	0	0	0
395890C	1	Leela's bab	6	5	2	0	1	1	0	0	0	0	0	0	0	0	0	0	1
400700D	0	Khutaija's b	1	2	1	0	1	4	0	0	0	0	0	0	0	0	0	0	0
403404D	1	Amit Bugali	31	6	7	0	1	2	0	0	0	0	0	0	1	1	0	0	1
404600C	0	Shaikh Subz	6	5	2	0	4	4	1	0	0	0	0	0	1	0	0	1	0
408915C	0	Baby's baby	6	5	2	0	1	3	0	0	0	0	0	0	0	0	0	0	0
412364C	0	Rebeca	9	5	2	0	1	4	0	0	0	0	0	0	0	0	0	0	1
415873C	0	Nibil	45	6	8	business	4	2	0	0	0	0	0	0	0	0	0	0	0
428707D	0	Subrata	46	6	8	business	4	2	0	0	0	0	0	0	0	0	0	0	0
436643D	0	Sudhir	27	6	8	0	3	2	0	0	0	0	0	0	0	0	0	0	0
443303D	0	Harish	6	5	2	0	1	3	0	0	0	0	0	0	1	0	0	0	1
446540D	0	Sweta	23	6	3	0	1	2	0	0	0	0	0	0	0	0	0	0	0
448850C	0	Jayakumari	55	6	8	housewife	1	2	0	0	0	0	0	0	0	0	0	0	0
448850c	0	Jayakumari	55	6	8	housewife	1	2	0	0	0	0	0	0	0	0	0	0	0
450147D	0	Teja	5	5	2	0	4	4	0	0	0	0	0	0	0	0	0	0	0
451026B	0	Renugopal	36	6	7	0	1	2	0	0	0	0	0	0	0	0	0	0	0
451052D	0	Hannah	1	2	1	0	1	3	0	0	0	0	0	0	0	0	0	0	0
451355D	1	Lakshman J	33	6	6	0	4	4	0	0	0	0	0	0	0	0	0	0	0

451741C	0	Akhtar	48	6	8	housewife	1	4	0	0	0	0	0	0	1	0	0	1
451793D	1	Farjhaha	15	5	2	0	1	4	0	0	0	0	0	0	1	0	0	0
453621D	0	Ganesh	27	6	6	0	1	4	0	0	0	0	0	0	0	0	0	0
453621d	0	Ganesh	43	6	6	0	1	3	0	0	0	5	0	0	1	0	0	0
481555C	0	Fazil	5	5	2	0	1	4	1	0	0	0	0	0	1	0	0	1
481555C	0	Fazil	6	5	2	0	1	4	0	0	0	0	0	0	1	0	0	0
484343D	0	Devi's baby	1	2	1	0	1	3	0	0	0	0	0	0	0	0	0	0
488238D	0	Sendhamar	61	7	6	0	2	2	0	0	0	7	0	CML	0	0	0	1
491857D	0	Anandhi	59	6	8	housewife	1	4	0	0	0	7	1	pemphigus	0	0	0	1
499070C	0	Abhishek	6	5	2	0	1	3	1	0	0	0	0	0	1	0	0	0
499600C	0	Kavitha's ba	6	5	2	0	1	2	0	0	0	0	0	0	0	0	0	0
501496D	0	Velankanni	5	5	1	0	1	4	0	0	0	0	0	CHILD synd	1	0	0	1
504309B	0	Sasikumar	33	6	7	0	1	2	0	0	0	0	0	0	0	0	0	0
504309b	0	Sasikumar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
504826D	0	Abdul	57	1	6	0	4	4	0	0	0	1	0	0	0	0	0	0
507481D	0	Bijoy Dey	24	6	3	0	4	4	0	0	3	0	0	0	0	0	0	0
507814D	0	Soni Kumar	10	5	2	0	1	3	0	0	0	0	0	0	0	0	0	0
512330D	0	Tsering	29	6	8	monk	4	1	0	0	0	0	0	0	0	0	0	0
512681D	0	Kavin	1	2	1	0	1	3	0	0	0	0	0	Atopic dern	0	0	0	0
514615D	0	Geetha's ba	1	2	1	0	1	3	0	0	0	0	0	0	0	0	0	0
515619D	0	Joydev	42	6	8	business	4	2	0	0	0	0	0	0	0	0	0	0
515792b	0	Pratheesh	33	6	8	doctor	1	2	0	0	0	0	0	0	0	0	0	0
515794D	0	Sneha	23	6	3	0	4	1	0	0	0	0	0	0	0	0	0	0
516021D	1	Joy	42	6	6	0	1	4	0	0	0	5	0	0	1	0	0	0
516439D	1	Chandraha	17	6	2	0	4	3	0	0	0	8	0	Nekam's dis	1	0	0	0
516856C	0	Deepalaksh	5	5	2	0	1	3	0	0	0	0	0	0	0	0	0	0
516995C	0	Sathiya Nir	4	4	1	0	1	4	0	0	0	0	0	0	1	0	0	0
517849C	0	Arumugasa	41	6	6	0	1	4	0	0	0	0	0	0	0	0	0	1
519029D	0	Kiran Kuma	26	6	3	0	4	3	0	0	0	0	0	0	0	0	0	0
519481B	0	Karthga De	37	6	7	0	1	3	0	0	0	0	0	0	0	0	0	0
520864D	0	Amit	5	5	2	0	4	3	0	0	0	0	0	0	1	0	0	0
522444D	0	Sakuntala	50	6	8	housewife	1	2	0	0	0	1	0	0	0	0	0	0
522653D	0	Haneef	57	6	6	0	4	4	0	0	0	0	0	0	1	0	0	0
523419D	0	Nelson	10	5	2	0	1	4	1	0	0	0	0	0	1	1	0	0
523440D	0	Chandana I	55	6	8	housewife	1	2	0	0	0	0	0	0	0	0	0	0
523776D	0	Paritosh	57	6	6	0	4	4	0	0	0	0	0	0	0	0	0	0
524351D	0	Ratan	60	6	8	farmer	4	3	0	0	0	8	1	SCC	1	0	0	1
524960D	0	Lambor	31	6	8	service	4	3	0	0	0	0	0	0	1	0	0	0
525099D	0	Kartik	3	4	1	0	4	3	0	0	0	0	0	atopic dern	0	0	0	0
525765D	0	Selvaraj	46	6	6	0	1	4	0	0	0	0	0	0	0	0	0	0
527336D	0	Sankha	5	5	2	0	4	2	0	0	0	0	0	0	0	0	0	0
527956D	0	Ganesh Ch	19	6	3	0	4	2	0	0	0	0	0	0	0	0	0	0
529336D	0	Sova Rani	59	6	8	housewife	4	3	0	0	0	0	0	0	0	0	0	0
530127D	0	Ramanatha	59	6	8	clerk	1	3	0	0	0	8	0	psoriasis	0	0	0	0
531370D	0	Ramprasad	31	6	6	0	4	4	0	0	0	0	0	0	0	0	0	0
531859D	0	Saroj	4	4	1	0	4	4	0	0	0	0	0	0	1	0	0	2
533253D	0	Anandan	46	6	6	0	1	4	0	0	0	0	0	0	1	1	0	0
533257D	0	Kenaram M	51	6	6	0	1	3	0	0	0	0	0	0	1	0	0	1
534482D	0	Bijaya	48	6	8	housewife	4	3	0	0	0	0	0	0	0	0	0	0
534535C	0	Dhaniksha	4	4	1	0	1	3	0	0	0	0	0	0	0	0	0	0
536767D	0	Dinakaran	27	6	6	0	1	4	0	0	0	0	0	0	0	0	0	0
537005D	0	Sahdeo	13	5	2	0	4	3	0	0	0	0	0	atopic dern	1	0	0	0
537704D	0	Saurav	33	6	7	0	1	1	1	0	0	0	0	0	0	0	0	1
538551B	0	Vignesh	11	5	2	0	1	4	1	0	0	0	0	0	1	0	0	0
541347D	0	Sai Geethik	23	6	3	0	4	1	0	0	0	0	0	0	0	0	0	0
541557D	0	Ram Ekbal	38	6	8	driver	4	3	0	0	0	0	0	0	0	0	0	0
542349D	0	Natarajan	49	6	8	business	1	2	0	0	0	5	0	0	1	0	0	0
542355D	0	Saranya	5	5	1	0	3	3	0	0	0	0	0	0	0	1	0	0
543224D	1	Gopalkrishr	26	6	3	0	1	1	0	0	0	0	0	arthritis un	0	0	0	0
543282D	0	Manjula	39	6	8	housewife	1	1	0	0	0	0	0	0	0	0	0	0
543349D	0	Rita	62	7	8	housewife	4	3	0	0	0	1	1	hypertensic	0	0	0	0
543655D	0	SukantA	24	6	3	0	4	3	0	0	0	0	0	0	0	0	0	0
544570D	0	Keithsfield	30	6	7	0	1	3	0	0	0	0	0	0	0	0	0	0
545791D	0	Rupa Shaw	33	6	8	teacher	1	2	0	0	0	0	0	0	0	0	0	0
547059C	0	Sanjay	5	5	2	0	1	4	0	0	0	0	0	0	0	0	0	1
547074D	1	Deepak Kur	26	6	3	0	4	3	0	0	0	0	0	0	0	0	0	1
547077D	0	Abha Kuma	24	6	3	0	4	2	0	0	0	0	0	0	0	0	0	0
548190D	0	Naveen Kur	37	6	8	business	4	3	0	0	0	0	0	0	0	0	0	0
550528D	0	satyen	45	6	8	clerk	4	3	0	0	0	0	0	0	0	0	0	0
550792A	0	Thayaramm	74	7	8	housewife	1	1	0	0	0	1	0	0	0	1	0	0
550867b	0	Kalaiselvi	36	6	8	housewife	1	4	0	0	0	0	0	0	0	0	0	0
553369D	0	Sankar	23	6	3	0	1	1	0	0	0	0	0	0	0	0	0	0
555187D	0	Shahinuzza	34	6	8	business	4	3	0	0	0	0	0	0	0	0	0	0
555662D	0	Dorairaj	81	7	8	retired	1	1	0	0	0	0	0	0	0	0	0	0
555666D	0	Saren	5	5	2	0	4	4	1	0	0	0	0	0	1	0	0	1
557888D	0	Sukla	46	6	8	housewife	4	2	0	0	0	0	0	0	0	0	0	0
560979D	0	Biplab	35	6	8	clerk	4	2	0	0	0	0	0	0	1	0	0	1
560979D	0	Biplab	46	6	8	business	4	3	0	0	0	0	0	0	0	0	0	0
562700d	0	vijaya	33	6	7	housewife	2	2	0	0	0	0	6	0	1	1	2	1
564990D	0	Anju	10	5	2	0	4	2	0	0	0	0	1	osteomyeli	0	0	0	1
565843D	0	Prasanta	34	6	6	0	4	4	0	0	0	0	0	0	0	0	0	1
569637B	0	Achintya	45	6	8	engineer	4	1	0	0	0	7	1	pemphigus	0	0	0	1
570972D	0	Goverdhan	29	6	8	business	3	2	0	0	0	0	0	0	1	0	0	0
573529D	0	Jeyachandr	56	6	6	0	4	4	0	0	0	0	0	0	0	0	0	0
574534D	0	Ashish	43	6	8	business	4	3	0	0	0	0	0	0	0	0	0	0
576633D	0	Namrada	30	6	8	housewife	1	2	0	0	0	0	0	0	0	0	0	0
576831D	0	Satan Roy	48	6	8	business	4	3	0	0	0	1	0	0	0	0	0	0
576995D	0	Satyanaray	37	6	6	0	4	3	0	0	0	0	0	0	0	0	0	0
578693B	0	Kavitha	12	5	2	0	1	4	0	0	0	0	0	0	0	0	0	0
581160D	0	Sheila	46	6	8	housewife	4	2	0	0	0	7	0	0	1	0	0	1
583001A	0	Rajakumar	24	6	7	0	1	1	0	0	0	0	0	0	0	0	0	0
585536D	0	Kasturi	38	6	8	housewife	4	1	0	0	0	7	1	pemphigus	0	0	0	1
585815D	0	Sulendar	46	6	8	business	4	2	0	0	0	0	0	0	1	0	0	1
589015D	0	Riya	2	4	1	0	4	3	0	0	0	0	0	0	0	0	0	0
590540D	0	Ravi Shank	51	6	6	0	1	4	0	0	0	0	0	0	0	0	0	0

590713D	0	Tapan	22	6	3	0	4	3	0	0	0	0	0	0	0	0	0	0
592097D	0	Shyamalen	62	7	8	retired	4	2	0	0	0	0	1	0	0	0	0	0
593568D	0	Sahada	14	5	2	0	1	2	0	0	0	8	0	psoriasis	0	0	0	0
594295D	0	Rao Sethun	49	6	8	business	1	1	0	0	0	0	0	0	0	0	0	0
595674D	0	NIvedita	22	6	3	0	4	1	0	0	0	0	0	0	1	1	0	1
601540B	0	Vijayalaksh	41	6	8	housewife	1	2	0	0	0	1	0	seizure disc	0	0	0	0
604477b	0	Saravanan	0	6	8	clerk	1	2	0	0	0	0	0	0	0	0	0	0
604618C	0	Anand	30	6	7	0	1	1	0	0	0	0	0	0	0	0	0	0
608805D	0	Usha	65	7	8	housewife	4	1	0	0	0	1	0	0	0	0	0	0
609892d	0	Bhagyalaks	65	7	8	housewife	4	2	0	0	0	0	0	0	0	0	0	0
611317D	0	Bidyut	35	6	6	0	4	4	0	0	0	8	0	Hansen's di	1	0	0	0
611493D	0	Dhanush	6	5	2	0	1	4	0	0	0	8	0	atopi derm	1	0	0	0
612150D	1	Ratan	25	6	3	0	4	4	0	0	0	0	0	0	1	0	0	0
612428D	1	Ranga Redc	61	7	8	business	4	1	0	0	0	0	0	0	0	0	0	0
612634D	0	Anuka Rai	42	6	8	0	4	3	0	0	0	0	0	0	0	0	0	0
615021C	0	Sanjay	8	5	2	0	1	3	0	0	0	0	0	0	0	0	0	0
632479D	0	Subya Safe	1	1	1	0	1	4	0	0	0	0	0	0	0	0	0	0
633854D	0	Rajendran	53	6	8	farmer	1	2	0	0	0	0	0	0	0	0	0	1
635762D	0	Preetha	8	5	2	0	3	2	0	0	0	7	1	SLE	0	0	0	0
635895d	1	Pooja	1	2	1	0	2	4	0	0	0	0	1	0	0	0	0	0
639624D	1	Sharoon	15	5	2	0	1	4	0	0	0	0	0	0	1	0	0	0
644191A	0	Vidya	23	6	3	0	1	2	0	0	0	0	0	0	0	0	0	0
644415C	0	Maheshwa	4	4	1	0	1	4	0	0	0	0	0	0	0	0	0	0
644958C	0	Varsha	4	4	2	0	1	3	0	0	0	0	0	0	0	0	0	0
648234C	0	Haradhan	64	7	8	retired teac	4	2	0	0	0	1	1	DRESS	0	1	0	1
651703d	0	Sonali	4	4	1	0	4	4	0	0	0	7	1	0	0	0	0	1
654095D	0	Narayan	43	6	8	business	4	2	0	0	0	1	0	0	0	0	0	0
654457C	0	Saramma	65	7	8	housewife	1	1	0	0	0	0	0	0	0	0	0	0
655581d	1	Rajiv	20	6	3	0	4	1	1	0	0	0	0	0	0	0	0	0
656338D	0	Subodh Rar	65	7	8	retired	4	4	0	0	0	0	0	0	0	0	0	0
657051D	0	Trinayani	45	6	8	healthcare	4	2	0	0	0	0	0	0	0	0	0	1
659562D	0	Madhuri	22	6	3	0	4	2	0	0	0	0	0	0	0	0	0	0
660140D	0	Prafulla	47	6	8	0	4	4	0	0	0	0	0	0	1	0	0	1
661891C	0	Shanthi's ba	4	4	1	0	1	3	0	0	0	0	0	0	0	0	0	0
663649D	1	Lubna	7	5	2	0	1	4	0	0	0	0	0	0	1	0	0	1
665258D	0	Jainul	44	6	8	housewife	4	4	0	0	0	0	0	0	1	0	0	1
666385d	0	Rubon	0	0	2	0	4	4	1	0	0	0	0	atopic	0	0	0	0
667224D	0	Atanu	43	6	8	clerk	4	2	0	0	0	0	0	0	1	0	0	0
668693D	0	Sasikala	54	6	8	housewife	1	3	0	0	0	0	0	pemphigus	0	0	0	1
670921D	0	Aryan	2	3	1	0	4	3	0	0	0	0	0	0	0	0	0	0
674167D	0	Yuvaraj	1	2	1	0	1	4	0	0	0	0	0	0	0	0	0	0
674474D	0	Hemamalin	1	1	1	0	1	4	0	0	0	0	0	0	1	0	0	0
674574D	1	Pema Wan	43	6	8	teacher	4	2	0	0	0	0	0	0	1	0	0	0
675648d	0	Thangamm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
675711d	0	Piu bhanda	6	5	2	0	4	4	1	0	0	0	0	0	0	0	0	0
676246d	0	poonambal	64	7	6	0	1	4	0	0	0	8	1	ca larynx	0	0	0	0
681008D	0	Manoj	30	6	8	business	4	2	0	0	0	0	0	0	0	0	0	0
681254D	0	Asif	24	6	3	0	4	2	0	0	0	0	0	NHL	0	0	0	0
683403D	0	Abdul	1	2	1	0	1	4	0	0	0	0	0	0	0	0	0	0
684978D	0	Murlidhar	25	6	3	0	4	3	0	0	0	0	0	0	0	0	0	0
685178d	0	Janki devi	31	6	8	0	4	3	0	0	0	0	0	0	0	0	0	0
688360D	1	Gita Rani D	32	6	8	housewife	0	3	0	0	0	0	0	0	0	1	0	1
688763D	0	Surendra	46	6	6	0	1	2	0	0	0	0	0	0	0	0	0	0
688771d	0	Kuppamal	51	6	8	housewife	1	3	0	0	0	0	0	0	0	0	0	0
689990A	0	Anandan	41	6	7	0	1	1	0	0	0	0	0	0	1	1	0	0
690567d	0	Savithri	47	6	8	teacher	3	2	0	0	0	0	0	0	1	0	0	0
691383B	0	Malarvizhi	38	6	8	housewife	1	4	0	0	0	0	0	0	0	0	0	0
695349D	0	Ratna	47	6	8	housewife	4	2	0	0	0	0	0	0	0	0	0	0
696818D	0	Prabhavati	60	7	8	housewife	1	1	0	0	0	0	0	0	0	0	0	1
697249D	0	Wendy	22	6	7	0	4	1	0	0	0	0	0	0	0	0	0	0
698160D	0	Shriya	49	6	8	housewife	1	2	0	0	0	1	0	0	1	1	0	1
698814D	0	Khairul	27	6	6	0	4	3	0	0	0	0	0	0	0	0	0	1
699365D	0	Vevek	20	6	3	0	1	2	0	0	0	0	0	0	1	0	0	0
703075D	0	Hari	1	2	1	0	1	3	0	0	0	0	0	0	0	0	0	0
704035C	0	Arnab	7	5	2	0	4	3	0	0	0	7	1	ALL	0	0	0	1
704173D	0	Elongovan	30	6	8	business	3	2	1	0	0	0	0	0	0	0	0	0
706237B	0	Madhavi	22	6	8	housewife	4	4	0	0	0	0	0	0	0	0	0	0
706661D	0	Mohd Ghos	34	6	8	business	1	4	0	0	0	0	0	0	0	0	0	0
710364D	0	Durai	43	6	6	0	1	2	0	0	0	0	0	0	1	0	0	1
711488D	0	Deendayal	28	6	6	0	4	4	0	0	0	0	0	0	0	0	0	0
715438D	0	Krishnakum	45	6	8	weaver	2	3	0	0	0	0	0	0	1	0	0	0
715493D	0	Pradip	42	6	6	0	4	4	0	0	0	0	0	0	0	0	0	0
718055D	1	Bandana	31	6	8	housewife	4	4	0	0	0	0	0	0	1	0	0	1
722401D	0	Noorjahan	56	6	8	housewife	1	4	0	0	0	0	0	0	0	0	0	0
724111D	0	Bhuvanesh	41	6	8	housewife	3	3	0	0	0	7	1	Pemphigus	0	1	0	0
727073D	0	Pintu	25	6	3	0	4	4	0	0	0	0	0	0	0	0	0	0
727844D	0	Nitaipada M	66	6	8	teacher	4	3	0	0	0	0	0	0	0	0	0	0
733511C	0	Praveen	49	6	8	clerk	4	2	0	0	0	0	0	0	0	1	0	0
736722A	0	Nissy	49	6	7	0	1	2	0	0	0	0	0	0	1	0	0	0
738973B	0	Lalitha	56	6	8	housewife	3	1	0	0	0	1	0	0	0	0	0	0
742294B	0	Soundaryar	86	7	8	retired	1	1	0	0	0	0	0	0	0	0	0	0
750229c	0	Dhanjit	38	6	6	0	4	4	0	0	0	0	0	0	0	0	0	0
762700D	0	Jeevitha	14	5	2	0	1	2	0	0	0	0	0	0	0	0	0	0
766290B	0	Christus	44	6	8	Business	1	2	0	0	0	0	0	0	0	0	0	0
771109	1	Roselyn	56	6	7	0	1	3	0	0	0	0	0	0	0	0	0	0
778841B	0	Ahiya	57	6	8	clerk	4	2	0	0	0	0	0	0	0	0	0	0
799822C	0	Dilip Kumar	56	6	8	Bank emplc	4	2	0	0	0	0	0	0	0	1	0	1
808254C	0	rAGHUL	4	4	1	0	1	4	1	0	0	0	0	0	0	0	0	0
809568C	0	Akash	4	4	1	0	1	3	0	0	0	0	0	0	1	0	0	0
816735c	0	Nithiyaksı	19	6	3	0	2	1	0	0	0	0	0	0	0	0	0	1
826635C	1	Vedhanaya	63	7	6	0	2	2	0	0	1	0	0	0	0	0	0	0
839946C	0	Ajith	4	4	1	0	1	4	1	0	0	0	0	0	0	0	0	0
852774A	0	Vasanthi	44	6	7	0	1	3	0	0	0	0	1	0	0	0	0	0
855266A	0	Krishnaveni	80	7	8	housewife	1	1	0	0	0	1	0	0	0	0	0	0
856907c	0	Divya	22	6	3	0	1	1	1	0	0	0	0	0	0	0	0	0

879426C	1	Rohith	4	4	1	0	1	4	1	0	0	0	0	0	1	0	0	0
881197b	0	Kannamma	35	6	8	housewife	1	4	0	0	0	0	0	0	0	0	0	0
890526A	0	Rajesh	20	6	3	0	2	2	1	0	0	0	0	0	0	0	0	0
895460C	0	Krishnan	56	6	6	0	1	3	0	0	0	0	0	0	0	0	0	0
897561B	0	Singhasini c	62	7	8	housewife	4	3	0	0	0	0	0	0	0	0	0	0
916539	0	Beulah	57	6	8	housewife	1	3	0	0	0	1	0	0	0	1	0	1
922773	0	Luka	24	6	6	0	1	4	0	0	0	0	1	0	0	0	0	1
922773	0	Luka	25	6	7	0	1	4	1	0	0	0	0	0	1	0	0	0
929130C	0	Shanthi's b	3	4	1	0	1	4	0	0	0	0	0	0	0	0	0	0
936302A	0	Ravikumar	49	6	8	business	1	1	0	0	0	0	0	0	0	0	0	0
937424b	0	Udaya babu	28	6	6	0	1	3	0	0	0	0	0	0	0	0	0	0
954071B	0	Thilagam	66	7	8	housewife	1	1	0	0	0	5	0	0	1	0	0	0
956917C	0	Debnath M	28	6	8	Bank emplc	4	2	0	0	0	0	0	0	0	0	0	0
974452b	0	Arputhama	49	6	8	housewife	4	2	0	0	0	0	0	0	0	0	0	0

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historyofre	highestedu	occupation	highestinco	sestatus	durationof	historyofpy	previouspy	otherrecurr	pain	burn	itch	asymptoma	typeofpyod	morphologi	otherpyode	site	size	number
0	2	3	1	2	14	1	0	0	-1	0	0	0	1	5	0	1	4	1
0	1	1	1	1	5	3	4	0	-1	0	0	0	1	5	0	4	4	2
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0	1	1	2	2	5	1	0	0	0	0	0	-1	1	6 `		10	2	2
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0	2	2	3	3	2	1	0	0	-1	0	-1	0	1	1	0	3	1	4
0	3	4	4	4	7	1	0	0	-1	0	0	0	2	0	0	0	0	0
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0	3	3	3	3	6	2	1	0	0	0	0	0	2	0	0	0	0	0
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0	2	3	2	2	7	1	0	0	-1	0	0	0	1	3	0	4	3	0
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0	6	6	6	4	10	1	0	0	-1	0	0	0	1	8 ecthyma		10	4	2
0	2	3	1	2	30	1	0	0	-1	0	0	0	1	2	0	7	3	3
0	4	4	3	3	30	1	0	0	-1	0	0	0	2	0	0	0	0	0
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surroundin	deeperinvo	necroticcha	lymphangit	lymphade	fever	secondaryp	othersecon	adiditionali	uniquekey	recstatus	lymphade	deeperinvo	necroticcha	lymphangit	fever1	secondaryp	othersecon	adiditionali
0	0	0	0	0	0	0	0	0	25	1	1	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	post melan
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0	0	0	0	0	0	0	0	0	84	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	103	1	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	111	1	0	0	0	0	0	0	hidradenitis	0
1	0	0	0	0	0	0	0	0	112	1	1	0	0	0	1	0	0	0
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0	0	0	0	0	0	5	0	0	118	1	0	0	0	0	0	0	infected er	Epidermoly
1	0	0	0	0	0	0	0	0	119	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	120	1	1	0	0	1	0	0	0	0
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1	0	0	0	0	0	0	0	0	127	1	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	3	0	0	177	1	0	0	0	0	0	0	0	infected bio	
0	0	0	0	0	0	2	0	0	178	1	0	0	0	1	0	0	stasis derm	0	
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0	0	0	0	0	0	5	0	0	197	1	0	0	0	0	0	0	subcorneal	0	
1	0	0	0	0	0	0	0	0	199	1	0	0	0	0	0	0	0	0	
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1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	
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1	0	0	0	0	0	0	0	0	211	1	0	0	0	0	0	0	0	0	
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1	0	0	0	0	0	0	0	0	213	1	0	1	0	0	0	0	0	0	
1	0	0	0	0	0	0	0	0	214	1	0	0	0	0	0	0	0	0	
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0	0	0	0	0	0	3	0	0	216	1	0	0	0	0	0	0	0	Hansen's ul
0	0	0	0	0	0	0	0	0	217	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	5	0	0	218	1	0	0	0	0	0	0	psoriasis wi	0
1	0	0	0	0	0	0	0	0	219	1	0	1	0	0	0	0	0	0
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0	0	0	0	0	0	5	0	0	221	1	0	0	0	0	0	0	TEN	0
1	0	0	0	0	0	0	0	0	7	1	0	0	1	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	230	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	231	1	0	1	0	0	1	0	0	0
1	0	0	0	0	0	0	0	0	232	1	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	5	0	0	237	1	0	0	0	0	0	0	DRESS with	0
0	0	0	0	0	0	2	0	0	17	1	0	0	0	0	0	0	0	scabies
1	0	0	0	0	0	0	0	0	238	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	2	0	0	239	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	240	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	9	1	0	0	0	0	0	0	atopic	
1	0	0	0	0	0	0	0	0	247	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	4	0	0	248	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	249	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	250	1	0	1	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	251	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	252	1	0	1	1	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	26	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	3	0	0	5	1	0	0	1	0	0	0	decubitus u	
1	0	0	0	0	0	0	0	0	253	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	254	1	0	1	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	255	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	6	1	1	1	0	0	1	0	0	0
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1	0	0	0	0	0	0	0	0	258	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	10	1	0	0	1	1	0	0	0	0
1	0	0	0	0	0	0	0	0	260	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	261	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	262	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	263	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	264	1	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	3	0	0	271	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	272	1	0	1	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	282	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	5	0	0	283	1	0	0	0	0	0	0	TEN	0
0	0	0	0	0	0	2	0	0	284	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	28	1	0	0	1	0	0	0	0	0
0	0	0	0	0	0	2	0	0	285	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	286	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	287	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	3	0	0	288	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	39	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	289	1	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	290	1	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	292	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	20	1	0	0	1	0	0	0	0	0
1	0	0	0	0	0	0	0	0	294	1	0	1	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	45	1	0	1	0	0	1	0	0	0
0	0	0	0	0	0	5	0	0	16	1	0	0	0	0	0	0	psoriasis	0
1	0	0	0	0	0	0	0	0	46	1	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	299	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	300	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	301	1	0	0	0	0	0	0	0	0

staphaureu	cons	strep	pseudomor	enterococc	klebsiella	morganella	proteus	otherbacte	mssa	betalactam	macrolide	quinolones	tetracycline	cotrimoxazo	linezolid	others	topicalfucid	topicalclind
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
1	0	1	0	0	0	0	0	E.coli	1	1	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	citrobacte	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	0
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1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	NFGNB	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	e.coli	0	0	0	0	0	0	0	0	1	0
1	0	0	0	0	0	0	0	NFGNB	1	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	No growth	0	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0
0	0	0	0	0	0	0	0	contaminar	0	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0
1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	contaminar	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	no growth	0	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	1	E.coli	0	0	0	0	0	0	0	0	1	0
1	0	0	0	0	1	0	0	E.coli	0	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0
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1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	Enterobact	1	1	1	1	1	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	no growth	0	0	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	citrobacte	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	No growth	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	enterobact	0	0	0	0	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	E.coli	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	no growth	0	0	0	0	0	0	0	0	0	0
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1	0	1	0	0	1	0	0	enterobact	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	1	E.coli	0	1	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	enterobact	0	1	1	0	0	1	0	0	0	0
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1	0	0	0	0	0	0	0	NFGNB	0	0	0	0	0	0	0	0	0	0
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0	1	0	0	0	0	0	0	NFGNB	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	E.coli	0	1	0	1	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	No growth	0	1	0	0	0	0	0	0	0	0
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0	0	1	0	0	0	0	0	E.coli	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	No growth	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	No growth	0	1	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	No growth	0	0	0	0	0	0	0	0	0	0
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1	0	1	0	0	0	0	0	NFGNB	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	M.tubercul	0	0	0	0	0	0	0	0	0	0
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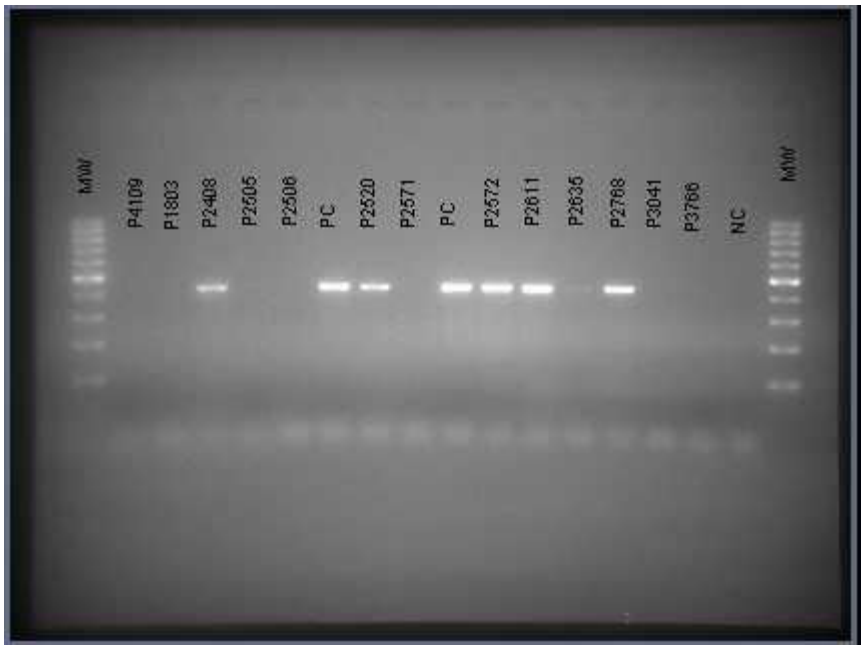
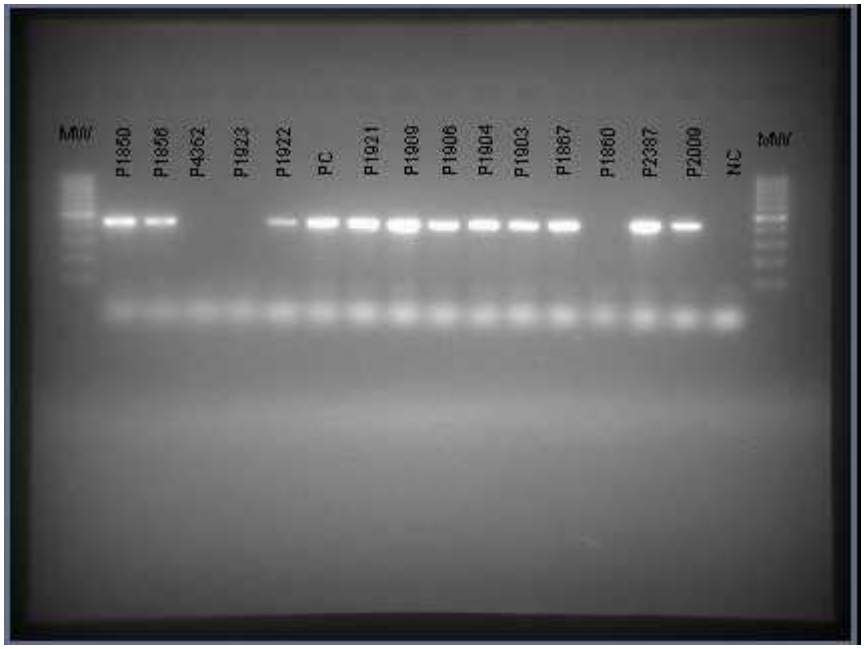


Pic 1: Gel run for PVL following PCR showing 5 positive strains



Pic 2: Gel run following PCR showing 11 PVL positive strains

(Key PC: positive control, NC: negative control)