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Evaluation of Resistance Pattern and Plasmid Profile of *Staphylococcus* Species Isolated from Clinical and Community Samples in Ibadan South-West, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors COE and OEF conceived and designed the experiments. Author COE performed the experiments. Authors OEF, SIS and AAO supervised the experiment. Authors COE, SIS and AAO analyzed the data. Author COE wrote the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Staphylococcus* species have been a major human pathogen of public health importance globally. This study was designed to evaluate the resistance pattern and plasmid profile of *Staphylococcus* species isolated from clinical and community settings.
Methodology: *Staphylococcus* species from clinical (55) and community (53) which were previously isolated in University of Ibadan and her teaching hospital and identified as *S. epidermidis* (92.6%), *S. aureus* (6.5%) and *S. xylosus* (0.9%) were used. The antibiogram and plasmid profiles were determined by standard procedures.

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Results: In clinical isolates of *S. epidermidis*, 30.9, 34.5, 40.0, 41.8, 60.0, 76.4, and 89.1% were resistant to chloramphenicol (CHL), streptomycin (STR), erythromycin (ERY), gentamycin (GEN), tetracycline (TET), cotrimoxazole (COT), and cloxacillin (CXC) respectively. Correspondingly, in community isolates of *S. epidermidis*, 28.3, 32.1, 50.9, 26.4, 58.5, 90.6 and 92.5% were resistant to these antibiotics. The only clinical *S. xylosus* isolated was resistant to all the antibiotics except CHL and STR. In the clinical isolates of *S. aureus*, 5.5, 5.5, 7.3, 7.3, 7.3, 9.1 and 9.1% were resistant to ERY, CHL, STR, GEN, TET, COT and CXC respectively. In community isolates, only one *S. aureus* was resistant to COT, CHL, ERY, GEN and STR while two were resistant to CXC. Plasmid profiling showed that 33/35 (94.3%) of clinical and 17/19 (89.5%) of community isolates had plasmid of size 23.13 kb.
Conclusion: The increasing resistance and similarity of plasmid profile of the community isolates to clinical isolates call for urgent establishment of antibiotic surveillance system to minimize the emergence of drug resistance pathogens in the community.

Keywords: Staphylococcus species; plasmid profile; resistance pattern.

1. INTRODUCTION

Staphylococcus species are Gram-positive, non-motile, non-sporing cocci occurring singly, in pair, and irregular clusters; colonies are opaque and may be white or creamy and are sometimes yellow or orange [1]. The genus *Staphylococcus* is pathogen of man and animals and colonizes the skin and mucosa membranes of their hosts [2]. Normally, they are grouped into two based their ability to clot blood plasma: coagulase positive staphylococci and coagulase negative staphylococci (CNS). *Staphylococcus* species have historically been a major human pathogen and continue to be one of the most commonly implicated bacteria causing human diseases throughout the world [3]. Its infection has become a global problem in both health institutions and community setting especially with the emergence of multi-drug resistant *Staphylococcus aureus*, MRSA [4].

Drug resistance has been an issue in the fight against bacterial infections. When new antibiotic is introduced into clinical practice, bacteria have been observed to resist such new drug after some months or years of continuous use [5-6]. The time of emergence and the rate of spread of resistant organisms can be unpredictable. Bacterial resistance occurs whenever the pathogens continue to reproduce at therapeutically attainable concentrations of the antibacterial agents. Resistance could occur extremely slow as observed with the resistance of *Staphylococcus* to neomycin, which was discovered only after nine years of clinical application [7]. Bacteria acquire resistance to antibiotics and other antibacterial agents either through chromosomal or extra chromosomal mediation [8]. Bacterial resistance of chromosomal origin was noticed shortly after the first antibiotics were put into large scale use, with the attendant indiscriminate administration of antibiotics and antibacterial agent [9].

The problem of bacterial resistance has been compounded with the discovery of various drug-inactivating enzymes in most bacteria. Notable among these enzymes are ß-lactamases, which act on susceptible antibiotics with cell wall acting activity and the transferases (O-phosphotransferases, O-adenyltransferases and N-acetyltransferases) with activity on certain aminoglycosides [10-11]. Most of these enzymes are coded by plasmid and plasmid mediated drug resistance in *Staphylococcus aureus* was reported specifically with gentamycin, tobramycin, kanamycin and chloramphenicol [12]. This discovery therefore, necessitated a shift of emphasis from a restrictive form of resistance mediated by extra chromosomal determinant. The eventual appearance of strains of staphylococci with multiple antibiotics significantly worsened this problem. This was found to involve different resistance genes linked to each other on segments of DNA capable moving from one bacterial cell to another by phenomena known as horizontal gene transfer [13-14].

It is a recurrent and noticeable phenomenon that drug resistance of bacteria in community occurs following its emergency in clinical settings. The information on resistance pattern and plasmid profile of *Staphylococcus* species in community setting is limited. This study was therefore, carried out in order to evaluate the resistance pattern and plasmid profiles of *Staphylococcus* species isolated from clinical and community settings (defined as all isolates outside clinical setting).

2. MATERIALS AND METHODS

2.1 Bacterial Isolates

Staphylococcus species previously isolated from clinical and community settings and identified using Restriction Fragment Polymorphism supplemented with PCR species-specific primers were used for the study. The bacteria were isolated between 2007 and 2011 from various clinical and community based samples which were stored in 60% glycerol at -80℃. Preliminary microbiological tests as growth on mannitol salt agar, Gram staining, catalase, coagulase were used to rescreen these isolates.

2.2 Sensitivity Test

An overnight broth culture suspension of each isolate was serially diluted with sterile distilled water until the turbidity matched 0.5 McFarland standard. This was inoculated onto a Mueller Hinton agar prepared plates and the antibiotic discs were distributed maintaining a distance of 30 mm edge to edge. The tests were interpreted after 24 h of incubation at 37℃. The diameter of the inhibition zones was measured using ruler and interpreted according to the criteria recommended by the CLSI [15].

2.3 Plasmid Isolation and Electrophoresis

Mini Prep method of Lech and Brent [16] was used as described below: Overnight broth culture of the organisms (1.5 ml) was transferred into eppendorff tubes and spanned for 1 minute at 13, 000 rpm. The supernatant was decanted and then vortexed to re-suspend the cells. About 300 µl of TENS solution (Tris 25 mM, EDTA 10 Mm, NaOH 0.1 N and SDS 0.5%) was added and mixed by inversion for 3-5 minutes until the solution became sticky. A volume of 150 µl of 3.0 M sodium acetate (pH 5.2) was added and vortexed. This was followed by spinning for 5minutes in a micro-centrifuge to pellet cell debris and chromosomal DNA. The supernatants were transferred to fresh eppendorff tubes and 900 µl of ice-cold absolute ethanol was added. This was spanned for another 10minutes to pellet plasmid DNA. The supernatants were discarded while the pellet was washed twice with 1 ml of 70% ethanol and dried. The pellet was re-suspended in 40 µl of distilled water. The extracted plasmid (10 µl) was resolved by 0.8% agarose gel electrophoresis.

3. RESULTS

3.1 Sources of Isolates

A total of 55 clinical *Staphylococcus* species were obtained of which *Staphylococcus epidermidis* from wound swabs accounted for 36.4%, eye swab (20.0%), semen (14.5%), and ear swab (10.9%). Sputum, throat, soft tissue and high vagina swabs each had one *S. epidermidis* (1.8%). Only urethral swab had *S. xylosus* (1.8%). In wound swabs, *S. aureus* (5.5%) were isolated while one *S. aureus* each was recovered from eye and ear specimens (Table 1). In community isolates (Table 2), *S. epidermidis* constituted the largest percentage (96.2%), with 71.70% recovered from human nostril, 17.0% in waste water, 1.9% in air, 1.9% on skin and 3.8% in private suite surfaces. One *Staphylococcus aureus* was isolated in both nostril and private suite surfaces.

Table 1. Distribution of clinical isolates according to their sources

Sources	*S. epidermidis*	*S. xylosus*	*S. aureus*
HVS	1	0	0
Semen	8	0	0
Ear	6	0	1
Eye	11	0	1
Soft tissue	1	0	0
Sputum	1	0	0
Throat	1	0	0
Urethra	0	1	0
Wound	20	0	3
Total	**49**	**1**	**5**

Table 2. Distributions of community isolates according to their sources

Sources	*S. epidermidis*	*S. xylosus*	*S. aureus*
Air	1	0	0
Water	9	0	0
Nostril	38	0	1
Skin	1	0	0
Private suite surface	2	0	1
Total	**51**	**0**	**2**

3.2 Antibiograms

In the clinical isolates of *S. epidermidis*, 30.9, 34.5, 40.0, 41.8, 60.0, 76.4, and 89.1% were resistant to Chloramphenicol (CHL), Streptomycin (STR), Erythromycin (ERY), Gentamycin (GEN), and Tetracycline (TET)

Cotrimoxazole (COT), and Cloxacillin (CXC) respectively. Correspondingly, in community isolates of *S. epidermidis*, 28.3, 32.1, 50.9, 26.4, 58.5, 90.6 and 92.5% were resistant to these antibiotics. In the clinical isolates of *S. aureus*, 5.5, 5.5, 7.3, 7.3, 7.3, 9.1 and 9.1% were resistant to ERY, CHL, STR, GEN, TET, COT and CXC respectively. In community isolates, 1.9% *S. aureus* were resistant to COT, CHL, ERY, GEN and STR while 3.8% were resistant to CXC. The only clinical *S. xylosus*, was resistant to all the antibiotics except CHL and STR (Table 3). Multiple resistance was also observed amongst the clinical and community isolates. In clinical isolates, 3 organisms were resistant to eight and seven different antibiotic classes while in community isolates, only two isolates were resistant to seven. Also, 13 and 8 clinical isolates were resistant to six and five antibiotic classes while 2 and 14 of community isolates were resistant the same number of antibiotics respectively. In the same way, 4 and 12 clinical and 10 and 18 community isolates were resistant to four and three different antibiotic classes respectively (Figs. 1 and 2).

3.3 Plasmid Profile and Analysis

Plasmid profiles of selected clinical (35) and community (19) isolates with multiple drug resistance showed that 94.3% of clinical and 89.5% of community isolates had plasmids of size 23.13kb (Fig. 3).

4. DISCUSSION

The evaluation of resistance pattern in this study reveals that high proportion of the isolates were resistant to most of the antibiotics in study. This was similar to few studies which reported increasing resistance of staphylococci to commonly used antibiotics [17-18]. This observation may not be surprising due to frequent abuse of most of these antibiotics especially those available from across the counter where they are sold with or without prescription in Nigeria [19]. Historically, resistance of clinical isolates of bacteria to antibiotics always outweigh that of community counterpart as a result of increasing antibiotic pressure. However, this study has shown that there are no significant difference in the resistance pattern between the clinical and community isolates to antibiotics tested except erythromycin and streptomycin. This is complete deviation from the earlier believe that community isolates have less tendency to develop resistance [20-22]. The implication of this is that community-acquired staphylococcal infection may be difficult to treat as the clinical

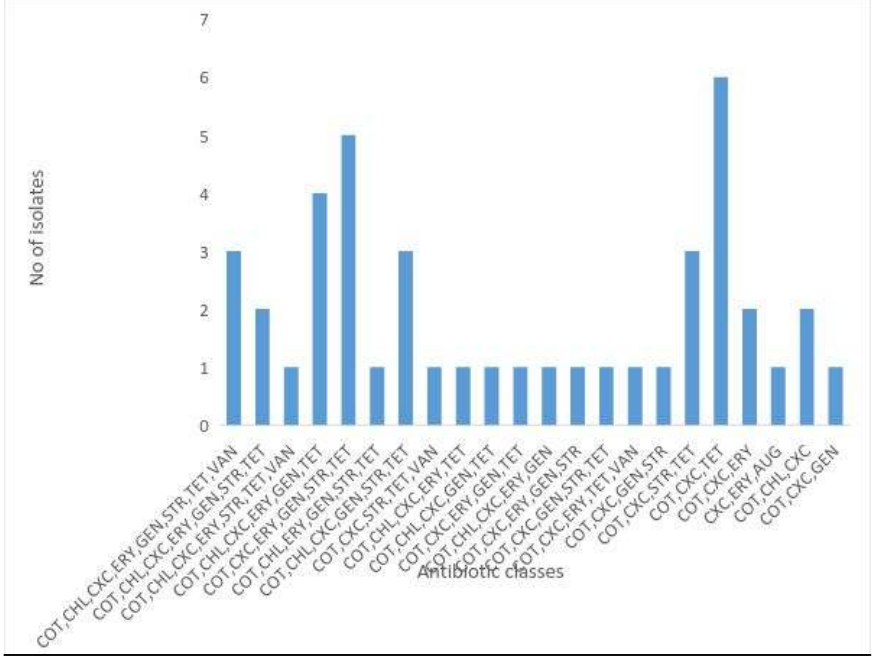

Fig. 1. Multiple resistance pattern among clinical isolates

Key: COT= Cotrimoxazole, STR =Streptomycin, CXC =Cloxacillin, TET =Tetracycline, ERY =Erythromycin, VAN =Vancomycin, GEN =Gentamicin, CHL= Chloramphenicol

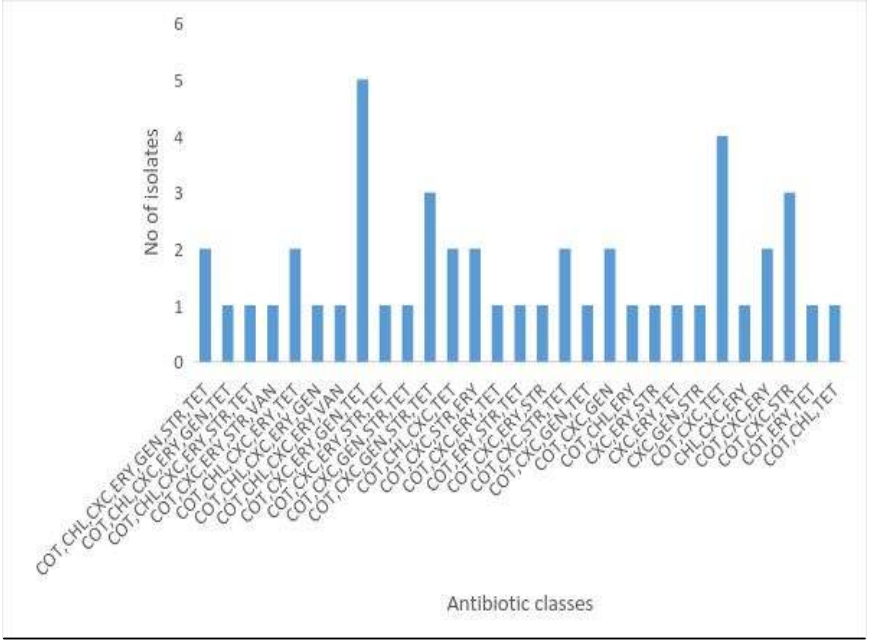

Fig. 2. Multiple resistance pattern among community isolates

Keys: COT= Cotrimoxazole, STR =Streptomycin, CXC =Cloxacillin, TET =Tetracycline, ERY =Erythromycin, VAN =Vancomycin, GEN =Gentamicin, CHL= Chloramphenicol

counterpart. Therefore, only a handful of antibiotics may be available for treatment of community associated infections. It is therefore, likely that there was drift of resistance genes from bacteria within the clinical settings to bacterial isolates in the community which become an important reservoirs in the spread of antibiotic resistance especially where indiscriminate use of antimicrobial agents and antibiotics are prevalent.

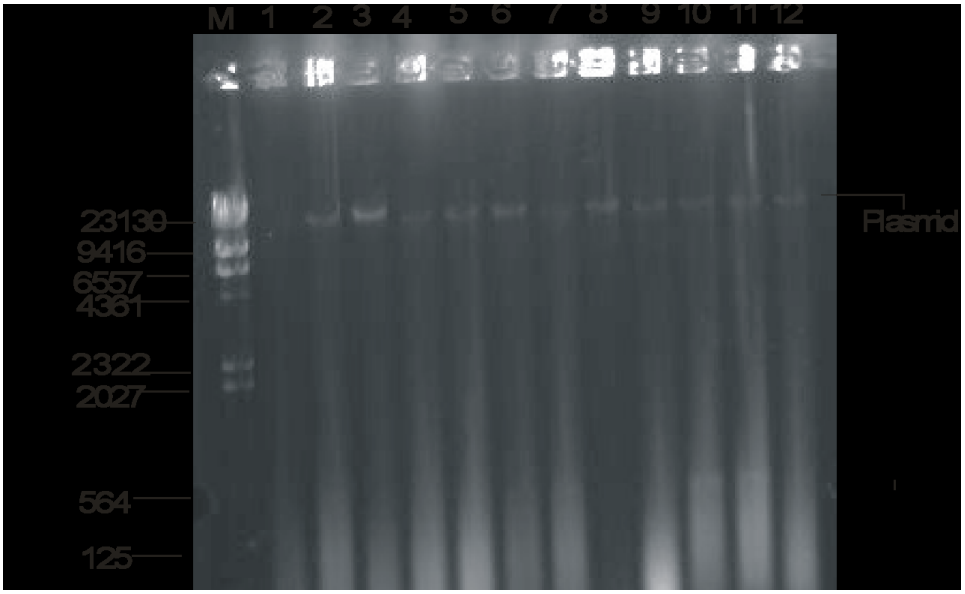

Fig. 3. Plasmid profiles of isolates of clinical and community isolates

Lane M=Molecular marker (Hind111 lambda DNA), 1=isolate without plasmid, 2-12= isolates with plasmids

Table 3. Comparison of percentage resistance of clinical and community isolates to various antibiotics

Antibiotics	Clinical (n=55)						Community (n=53)				*P*
	S. epidermidis	**%**	***S. aureus***	**%**	***S. xylosus***	**%**	***S. epidermidis***	**%**	***S. aureus***	**%**	
COT	42	76.4	5	9.1	1	1.8	48	90.6	1	1.9	0.457
CHL	17	30.9	3	5.5	0	0	15	28.3	1	1.9	0.411
CXC	49	89.1	5	9.1	1	1.8	49	92.5	2	3.8	-
ERY	22	40.0	3	5.5	1	1.8	27	50.9	1	1.9	0.025[a]
GEN	23	41.8	4	7.3	1	1.8	14	26.4	1	1.9	0.487
STR	19	34.5	4	7.3	0	0	17	32.1	1	1.9	0.019[a]
AUG	47	85.5	5	9.1	1	1.8	50	94.3	2	3.8	0.842
TET	33	60.0	4	7.3	1	1.8	31	58.5	0	0	0.296

Keys: P= level of significance (≤ 0.05), n= sample size, a= significant at 0.05

The multiple antibiotics resistance of coagulase negative staphylococci (CNS) observed in this study was similar to previous work [23] in which multiple resistance among the CNS reported to be as high as 80.77%. The antibiogram pattern in this study showed that *S. epidermidis* tends to be resistant to a wider range of antibiotics and this is consistent with a report in Lagos, Nigeria in which 77.0% of *S. epidermidis* were resistant [23]. The earlier review by Pfaller and Henweldt [24] indicates that *S. epidermidis* has become resistant to commonly used antibiotics which may serve as reservoir for antibiotic resistance strains in hospitals. These antibiotic resistant determinants can be transferred to new bacterial species as part of the large conjugative replicons which commonly code resistance to some aminoglycosides such as gentamycin, kanamycin [25]. The rising resistance profile of *S. xylosus* in this study was similar to a study of staphylococci associated with food and used in starter cultures in which these species (95%) were resistance to seven antibiotics [26]. The limited number of *S. xylosus isolated* hampered the overall scientific significance with respect to resistance. However, the resistance profile of *S. xylosus* has been previously documented [27]. The reason for the multiple antibiotic resistance in CNS is unknown, but transfer of genetic elements between CNS and *S. aureus* is a plausible cause. Also, CNS carries a variety of multiple resistance genes on their plasmid which can be exchanged and spread amongst different species of staphylococci including *S. aureus*.

The plasmid analysis showed that 23.13 kb plasmid was similar to 23.13 kb identified previously [28] which harbor resistance determinant to β-lactam antibiotics. In this study, the homogeneity of the isolates with respect to antibiograms and plasmid profiles is an evidence of genetic transfer from a common source and this is likely to have arisen through horizontal gene transfer from a single strain or its derivatives from hospital to hospital and hospital to community and verse versa. Evolutionary events through recombination or transposition might have resulted to emergence of these strains. The frequent use of antibiotics has led to selective pressure to emergence of resistance determinants within many staphylococci as evidenced by outbreak of resistance mostly encountered following its introduction into clinical practice. Geetha and others [23] reported the successful transfer of R-plasmid *in vivo* by mixed culture transfer on solid media. This signifies the epidemiological significance of normal staphylococcal habitat in the emergence of antibiotic resistance with topical use of antibiotics which predispose the organisms to antibiotic selective pressure for plasmid gene expression.

5. CONCLUSIONS AND RECOMMENDATION

The increasing resistance and similarity of plasmid profile of the community isolates to clinical isolates call for urgent establishment of antibiotic surveillance system to minimize the emergence of drug resistance pathogens in the community. In addition, staphylococcal infections have been associated with significant morbidity and mortality in health-care institutions, therefore, accurate analysis of resistance and plasmid profiles may allow for provision of better antimicrobial therapy and epidemiological surveillance. Besides, the similarity in resistance and plasmid patterns of clinical and community isolates of *staphylococcus* species implies that community associated infections should be treated as a matter of urgency as the clinical counterpart. There is need for proper clinical documentation of drift in resistance pattern of staphylococci especially with emergence of MRSA in this region. Setting up antibiotic surveillance system could reduce the spread of staphylococcal resistance in the community setting.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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