International Journal of Research in Medical Sciences
Sahu KK et al. Int J Res Med Sci. 2018 Jan;6(1):69-73
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20175554

Original Research Article

Surveillance of bacteria *Pseudomonas aeruginosa* and MRSA associated with acute suppurative otitis media (ASOM)

Kundan K. Sahu[1], Siba N. Rath[2], Rabindra N. Padhy[2], Rajashree Panigrahi[1]*

[1]Department of Microbiology, [2]Central Research Laboratory, IMS and Sum Hospital, S 'O' A University Kalinga Nagar, Bhubaneswar, Odisha, India

Received: 30 October 2017
Revised: 06 December 2017
Accepted: 07 December 2017

***Correspondence:**
Dr. Rajashree Panigrahi,
E-mail: dr.rajashreep@gmail.com

ABSTRACT

Background: Otitis media particularly with suppuration is a critical disease-causing perforation of the tympanic membrane associated with changes of the mucoperiosteum of the middle ear cleft. This surveillance includes isolation and antibiotic profiles of causative bacteria from ear discharges of patients in 3years attending outpatients of a hospital.
Methods: Bacterial strains were grown in suitable media and were subjected to antibiotic profiling by the Kirby-Bauer's method with most antibiotics of the day.
Results: In total there were 1164 colonies with 1043 bacterial and 121 fungal isolates from 1230 ear discharge samples. Among 371 *Pseudomonas aeruginosa* isolates, tobramycin 30 µg/disk had the highest susceptibility rate as 93.2%, followed by ceftazidime 30µg/disk 91.5% and amikacin 10µg/disk 64.4%. From 359 *Staphylococcus* isolates, there were 236 coagulase negative *Staphylococcus* (CONS) + methicillin sensitive *S. aureus* (MSSA) and 123 methicillin resistant *S. aureus* (MRSA). *Staphylococcus* including MRSA isolates were most susceptibility to cloxacillin 15µg/disk 95.2%, followed by erythromycin 15µg/disk 83.3% and gentamicin 30µg/disk 78.5%. Of 1164, 49 patients presented post aural abscess, 12 patients had intracranial complications, 9 patients had facial palsy and 3 patients had labyrinthitis.
Conclusions: Isolated bacteria, *P. aeruginosa* and MRSA were multidrug resistant. *P. aeruginosa* was most common followed by *S. aureus*. More than 90% *P. aeruginosa* and 90% *S. aureus* isolates were sensitive to tobramycin 30 µg/disk and cloxacillin 30 µg/disk, respectively. Therefore, these two antibiotics may be included in the formulary regimen to overcome bacterial infections involved in ASOM.

Keywords: Acute suppurative otitis media, Intracranial complications, *Pseudomonas aeruginosa*, MRSA

INTRODUCTION

The generic term, 'otitis media' includes widely, cases of 'acute otitis media' (AOM) and cases of 'otitis media with effusion' (OME); basically, these are non-suppurative. Moreover, 'chronic otitis media' (COM) is the gathering of pus from suppurations when infections are chronic; eventually, Acute suppurative otitis media (ASOM) are with inflammation and the production of pus.[1] Additionally, ASOM may remain inactive with the potential to be active occasionally, leading to a perforation of the tympanic membrane associated with changes of the mucoperiosteum of the middle ear cleft with/without mucoid or mucopurulent otorrhea.[1-3] It takes usually 2 or 3weeks or more duration, for the disease to be recognized as active. A healed COM may have permanent abnormalities of the pars tensa; but with an intact pars tensa the occurrence of COM is rare.[3] ASOM

could lead to hearing loss, intermittent otalgia causing psychological trauma. In ASOM, the most causative bacteria are Klebsiella species, Proteus species, *P. aeruginosa* and *S. aureus*.[4] And other bacteria commonly isolated from patients with AOM are *Haemophilusinfluenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*.[5] Moreover, *P. aeruginosa* had been seen as a notorious pathogen in this hospital too.[6] Mainly found in wounds and urinary tract, it finds ways as bloodstream infection (BSI) to innards causing comorbidities.[7] Indeed, the ability of these organisms to form biofilm may contribute to their frequency in ASOM.[8] As it is known, the rate of invasion of a pathogenic bacterium directly depends on its level of drug resistance, apart from immune-conditions of patients.[9]

Particularly, several clonal variants of *S. aureus* were resistant to the penicillin group of antibiotics, after which methicillin/oxacillin were introduced for the control. Subsequently, methicillin resistant *S. aureus* (MRSA), causing surgical site infections and wound emerged.[10] The most gruesome situation is that MRSA strains have emerged with concomitant/subsequent resistance to most commonly used antibiotics of groups, aminoglycosides, macrolides, fluoroquinolones, chloramphenicol and tetracycline and many more such as, to cephalosporins, cefems and other β-lactams, ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactamand the carbapenem, imipenem. Thus, MRSA isolates are MDR too.[11] Moreover, the most dominating fungal species were of *Candida* and *Aspergillus* along with MRSA; and in a surveillance, 50% patients were diagnosed with candidiasis.[10] Indeed, *Candida albicans* was originally a harmless fungus in healthy persons, but its causes superficial to life-threatening uncontrollablesystemic infections due to the emergence of antifungal resistance.[12]

This work describes surveillance of bacterial flora from ear discharges of patients attending the outpatient's department (OPD) of ENT department of the hospital, in the last 3years. And the cited two fungi were too isolated along with bacteria. Antibiograms of isolated bacterial taxa were determined to assess the spectrum of ASOM that would help in rescheduling antimicrobial stewardship program of the hospital or the zone of central Odisha.

METHODS

A total of 1230 pus discharges from clinically diagnosed ASOM cases were collected, during January 2012 to January 2015 with sterile cotton swab sticks. Pus swabs were cultured on blood and Mac Conkey agar plates that were incubated at 37°C overnight for pathogenic bacteria, which were identified according to the standard method used for bacteria and concomitantly for fungi.[6,13,14] Antibiotic susceptibility tests of isolated bacteria were done according to Clinical Laboratory Standard Institute guidelines, as described.[15,16] Standard antimicrobial discs (HiMedia, Mumbai) used for *S. aureus* were, oxacillin, cotrimoxazole, penicillin, cloxacillin, gentamicin, chloramphenicol, ciprofloxacin and vancomycin; similar discs used for *P. aeruginosa* were gentamicin, chloramphenicol, ciprofloxacin, ceftazidime, piperacillin, carbenecillin and tobramycin.

Antibiotic sensitivity and detection of MRSA

The standard ATCC strain and all the isolated *S. aureus* strains were subjected to antibiotic sensitivity tests with antibiotics, by the Kirby-Bauer's method (disc diffusion) detailed previously.[11] For the detection of MRSA, chromogenic agar media test was used; pure clinical isolates of *S. aureus* were streaked onto MRSA-agar media as described.11Muller-Hinton agar (MHA) plates were incubated at 37°C for 18hrs and inhibition-zone diameters were measured. A value of inhibition-zone diameter less than 22mm was reported as oxacillin resistant and that more than 22mm was considered as oxacillin sensitive.[11]

Identification of fungi

Direct microscopic examination of the cotton swab with samples was carried out by mounting sample lots treated with 1-2 drops of 10-20% KOH for 15-30min. Each specimen-lot was inoculated on two sets of Sabouraud's dextrose agar slopes, one set with chloramphenicol and the other set with cycloheximide (chloramphenicol-0.05mg/mL, cycloheximide-0.5mg/mL). Cultures were incubated at room temperature for 4-6weeks and were observed regularly for possible growth. Fungal isolates were identified on the basis of duration of growth and surface morphology of colonies, as well as pigment production on the reverse and microscopic examination of hyphae in lacto phenol cotton blue preparation.[6,16]

RESULTS

From 1230 collected samples, 1164 bacterial and fungal colonies grew as 629 single and 535 mixed colonies on agar plates and no microbial growth was seen with 66 samples. There were 1043 bacterial and 121 fungal isolates in total. The most common causal bacteria isolated were 220 isolates of *S. aureus* with and 188 isolates of *P. aeruginosa*; and 19 isolates of *S. aureus* were MRSA, and 64 isolates were coagulase negative *Staphylococcus* (CONS). Bacteria, *P. aeruginosa* was isolated in 183 of the total 1164 samples that yielded mixed colonies of *S. aureus*, Klebsiella species and Proteus species, followed by *E. coli*, given in Table 1. Fungi accounted for 63 isolates of Aspergillus species and 68 isolates of Candida species as both single and mixed colonies from1164 growth-yielding samples, given in Table 1.

Antibiograms of the most common bacteria, *P. aeruginosa* and *S. aureus* (other than MRSA) are depicted in Figure 1. Among *P. aeruginosa*, tobramycin

30µg/disk had the highest susceptibility rate as 93.2%, followed by ceftazidime 30µg/disk 91.5% and amikacin 10µg/disk 64.4%. And 95.2% *S. aureus* isolates were susceptible to cloxacillin 15µg/disk, followed by 83.3% isolates to erythromycin 15µg/disk and 78.5% isolates to gentamicin 30µg/disk, given in Figure 3. All isolates of MRSA were MDR; however, none of those isolates were resistant to vancomycin 30µg/disk.

Table 1: Growth of bacteria and fungi in cultures of ear discharge samples of OPD patients with ASOM as single colony and mixed colonies.

Organisms	Single colony isolates N_1=629 (100)	Mixed colony isolates N_2=535 (100)	Total isolates (n_1+n_2)= N=1164 (100)
Enterobacter species	19 (03.0)	-	19 (01.6)
Cons	64 (10.1)	-	64 (05.4)
MRSA	19 (03.0)	104 (19.7)	123 (10.5)
MSSA	137 (22.0)	35 (06.8)	172 (14.7)
E. coli	47 (07.7)	51 (09.8)	98 (08.4)
Citrobacter species	34 (05.4)	-	34 (02.9)
Klebsiella species	47 (07.7)	45 (8.7)	92 (07.9)
Proteus species	19 (03.0)	41 (7.7)	60 (05.1)
P. aeruginosa	188 (30.1)	183 (34.3)	371 (31.8)
Aspergillus species	33 (05.3)	30(05.8)	63 (05.4)
Candida species	22 (03.5)	46(08.8)	68 (05.8)

Note: CONS, coagulase negative Staphylococcus; MRSA, methicillin resistant *S. aureus*; MSSA, methicillin sensitive *S. aureus*; OPD, outpatients department; percent values are in parenthesis; N or total colonies= 1164, from the total 1230 samples; the rest 66 samples had no growth; there were 121(63+68) fungal isolates.

Of 1164 samples, 73 patients had complicated and 1091 patients had uncomplicated ASOM, as detailed: only 48 cases had as single bacterium isolated as a single colony, while the remaining 198 cases had two or more bacteria isolated as mixed colonies, given in Table 2.

Table 2: Numbers of growing organisms from cultures of ear discharge samples in patients with complicated and uncomplicated ASOM.

Types of organisms	Complicated ASOM	Uncomplicated ASOM	Total
As single colony	48 (0.05)	581 (0.49)	N_1=629 (0.54)
As mixed colonies	25 (0.02)	510 (0.43)	N_2=535 (0.45)
Total	73 (0.06)	1091 (0.94)	N=1164 (100)

Of the total 1230 patients, 49 had post aural abscess, 12 patients had intracranial complications, 9 patients presented with facial palsy and 3 patients presented with labyrinthitis, given in Table 3.

Table 3: Numbers of patients with complications as comorbidities causing ASOM in 3 years.

Year	Facial palsy	Intracranial complication	Post-aural abscess	Labyrinthitis	Total
2012	3	3	16	2	24
2013	2	4	18	0	24
2014	4	5	15	1	25
Total	9 (12.)	12 (16.6)	49 (67.1)	3 (3.3)	73 (10)

Note: Percent values are in parenthesis.

Furthermore, it was seen that the trend of intracranial complications was gradually decreasing while, intracranial complications were in an increasing trend, although there was no significant change in overall incidences of ASOM. From 359 *S. aureus* samples, a total of 123 MRSA strains and 236 strains of 'CONS + (MSSA)' (methicillin sensitive *S. aureus*) strains, as both single and mixed colonies were isolated. The minimum inhibitory concentration (MIC) range against oxacillin was 16 to 512µg/mL, the MIC range was 1 to 4µg/mL, for MRSA and 'CONS+MSSA'. These MIC values confirmed the presence of MRSA strains, as the break

point for being resistant to oxacillin was ≥4µg/mL, given in Table 4.

Table 4: Detection of MRSA and 'CONS+MSSA' isolates based on MIC values due to oxacillin in a 12x8 micro-titre plate.

Well	Oxacillin (µg/ml)	Number of isolates	
		MRSA =123	CONS+MSSA =236
1	0	123	236
2	≤0.25	–	–
3	0.5	–	–
4	1	–	83
5	2	–	75
6	4	–	78
7	8	–	–
8	16	23	–
9	32	26	–
10	64	27	–
11	128	29	–
12	≥ 256	28	–

Note: The oxacillin stock solution of 512µg/mL was serially diluted at each successive well, from the 12th well for final concentration of 0.25µg/mL oxacillin at the 2nd well; –, no growth; total *Staphylococcussp*. = MRSA with 123 + (CONS+MSSA) with 236=359 colonies. Results of the second repeated experiment are presented.

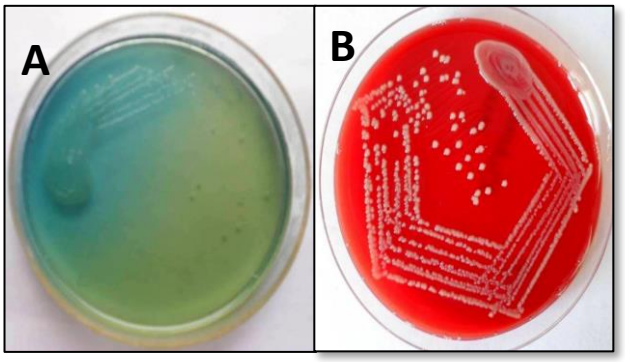

Figure 1: A) Confluent growth and isolated green colonies *of P. aeruginosa* on nutrient agar plate; and B) methicillin resistant colorless colonies of *S. aureus* (MRSA) on blood agar plate.

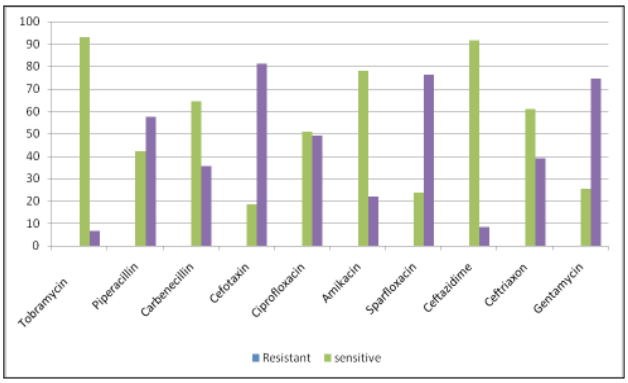

Figure 2: Antibiogram of *P. aeruginosa*.

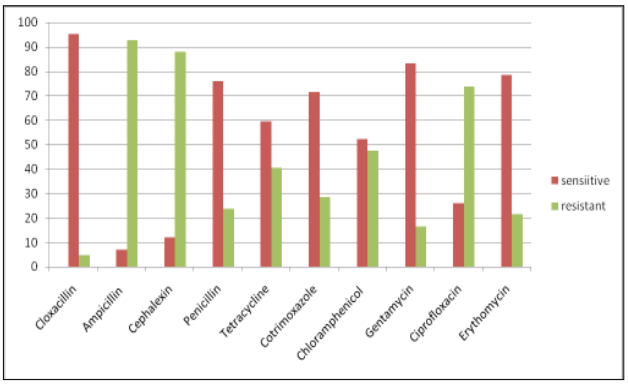

Figure 3: Antibiogram of *S. aureus*.

DISCUSSION

ASOM is a disease associated with the structural change in middle ear; and permanent abnormality of pars tensa or pars flaccid, mostly occur as sequelae of long standing middle ear effusion, inadequately treated AOM, eustachian tube dysfunction or even from a negative middle ear pressure. In the developing countries, poverty, ignorance, dearth of specialists and limited access to medical care amongst others conspire to worsen the occurrence and complications of ASOM; poor living conditions, poor access to medical care, inadequate medical treatment, recurrent upper respiratory tract infections and nasal diseases have been recognized as risk factors for ASOM.[17,18] Atticoantral disease most commonly is involved with the pars flaccida and posterior superior quadrant of pars tensa. It is characterized by the formation of a retraction pocket in which, keratin and desquamated epithelial debris accumulate to produce cholesteatoma; eventually it is considered to be a dangerous form of the disease because of the development of several intracranial and extracranial complications.[18]

Moreover, staphylococci are a part of the normal flora, but those remain invasive causing a variety of body infections. *S. aureus* is the most notorious nosocomial pathogen and OPD.[11]

Although the clinical relevance of CONS is still controversial, patients at risk of CONS infections include neonates, those with intravascular catheters, prosthetic devices and surgical wounds in immune-compromised individuals. The remarkable ability of *S. aureus* and CONS to acquire antibiotic resistance limits therapeutic options, attended with high rates of morbidity and mortality, including costs of hospitalization.[19]

Particularly, several clonal variants of *S. aureus* and MRSA are resistant to the penicillin group of antibiotics, methicillin/oxacillin. Moreover, in a German study, it was reported that a majority of MRSA strains were from wound infections (56.9%), with pneumonia cases being

the second most common (21.0%), followed by BSI (15.1%).[11]

CONCLUSION

Both *P. aeruginosa* and MRSA were amply detected from ear discharges from patients with ASOM. From antibiogram of *P. aeruginosa* isolates, tobramycin had the highest susceptibility rate as 93.2%, followed by ceftazidime 91.5% and amikacin 64.4%. And *S. aureus* including MRSA isolates were most susceptible to cloxacillin 30µg/disk 95.2%, followed by erythromycin 15µg/disk 83.3% and gentamicin 30µg/disk 78.5%. Of 1164, 49 patients presented post aural abscess, 12 patients had intracranial complications, 9 patients had facial palsy and 3 patients had labyrinthitis. This study revealed ciprofloxacin as less effective in the treatment of active ASOM. This report recoded that in this hospital, tobramycin and cloxacillin could be used to treat ASOM.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Berman S. Otitis media in children. N Eng J Med. 1995;23:1560-5.
2. Browning GG, Merchant SN, Kelly G, Swan IR, Canter R, McKerrow SW. Chronic Otitis Media. Scott-Brown's Otorhinolaryngology Head and Neck. Edward Arnold Ltd. 2008;19:3406-10.
3. Afolabi OA, Fadare JO, Omokanye HK, Olatoke F, Odi TO, Saka MJ, Adaranijo RK. Socioeconomic challenges of chronic suppurativeotitis media management in state tertiary health facility in Nigeria. E J ENT Sc. 2014;15:17-22.
4. Couzos S, Lea T, Mullar R. Effectiveness of ototopical antibiotics for ASOM in Aboriginal children, a community based multicenter double blind randomized controlled trial. Med J Aust. 2003;4:185-90.
5. Bluestone CD, Klein JO. In: Bluestone CD, Klein JO. Otitis media in infants and children. 3rd ed. Philadelphia PA, WB Saunders;2001:79-101.
6. Sahu MC, RN Padhy. Bayesian evaluation of two conventional diagnostic methods for pathogenic fungal infections. J Acute Med. 2014;4:109-19.
7. Askarian M, Hossseini R, Kheirandish P. Incidence and outcome of nosocomial infections in female burn patients in Shiraz, Iran. Am J Infect Control. 2004;32:23-6.
8. Ahmed B, Hydri S, Afridi AAK. et al. Microbiology of ear discharge in Quetta. J Coll Physician Surg Pak. 2005;15:583-4.
9. Giamarellos-Bourboulis EJ, Papadimitriou E, Galanakis N, Antonopoulou A, Tsaganos T, Kanellakopoulou K. Multidrug resistance to antimicrobials as a predominant factor influencing patient survival. Int J Antimicrob Agent. 2006;27:476-81.
10. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39:309-17.
11. Dubey D, Rath S, Sahu MC, Patnaik L, Debata NK, Padhy RN. Surveillance of infection status of drug resistant Staphylococcus aureus in an Indian teaching hospital. Asian Pacif J Trop Dis. 2013;3:133-42.
12. Richardson M, LasseFlorl C. Changing epidemiology of systemic fungal infections. Clin Microbiol Infect. 2008;14:5-24.
13. Rath S, Padhy RN. Surveillance of acute community acquired urinary tract bacterial infections. J Acute Dis. 2015;3:186-95.
14. Prajna L, Vijayakumar A. Atlas of fungal corneal ulcers clinical features and laboratory identification methods. New Delhi: Jaypee Brothers Medical Publishers; 2008.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. PA: Wayne; 2011.
16. Rath SN, Panda M, Sahu MC, Padhy RN. Bayesian analysis of two diagnostic methods for paediatric ringworm infections in a teaching hospital. J Mycol Médical. 2015;25:191-9.
17. Vikram BK, Khaja N, Udayashankar SG. Clinico-epidemiological study of complicated and uncomplicated chronic suppurative otitis media. J Laryngol Otol. 2008;122:226-42.
18. Chowdhury MA, Alauddin A. Comparative study between tubotympanic and atticoantral type of chronic suppurative otitis media. Bangladesh Med Res Counc Bull. 2002;1:36-44.
19. Zong Z, Peng C, Lu X. Diversity of SCC mec elements in methicillin resistant coagulase-negative staphylococci clinical isolates. PLoS One. 2011;5:191-200.

Cite this article as: Sahu KK, Rath SN, Padhy RN, Panigrahi R. Surveillance of bacteria Pseudomonas aeruginosa and MRSA associated with acute suppurative otitis media (ASOM). Int J Res Med Sci 2018;6:69-73.