Research Paper

Surveillance of antibiotic sensitivity pattern in chronic suppurative otitis media of an Indian teaching hospital

Mahesh Chandra Sahu [a], Santosh Kumar Swain [b],*

[a] IMS and SUM Hospital, Siksha "O" Anusandhan University, K8, Kalinganagar, Bhubaneswar, 751003, Odisha, India
[b] Department of Otorhinolaryngology, IMS and SUM Hospital, Siksha "O" Anusandhan University, K8, Kalinganagar, Bhubaneswar, 751003, Odisha, India

Abstract *Introduction:* Chronic suppurative otitis media (CSOM) is a common problem in worldwide and untreated CSOM leads to fatal complications like facial nerve paralysis, lateral sinus thrombosis, labyrinthitis, meningitis and brain abscess in developing country like India. *Objective:* To isolate causative bacteria and antibiotic sensitivity pattern for CSOM and to know the prevalence of extended spectrum beta lactamases (ESBL) and Metallobetalactamases (MBL) in CSOM patients.

Methods: A total of 500 ear swabs of clinical suspected CSOM patients were cultured on specific cultured medium and identified the bacteria with conventional methods. Then all the identified bacteria were subjected with specific antibiotics by the Kirby−Bauer's method to know the resistance pattern of antibiotics. ESBL and MBL strains were detected by double disc diffusion test.

Results: A total of 384 bacteria were isolated from 500 CSOM patients, among them 86 *P. aeruginosa* (22.40%), 112 *Staphylococcus aureus* (29.17%), 53 *A. baumannii* (13.80%), 32 *E. aerogenes* (18%), 26 *C. freundii* (6.77%), 24 *K. oxytoca* (6.25%), 23 *P. vulgaris* (5.99%), 18 *K. pneumoniae* (4.69%) and 10 *P. mirabilis* (2.60%) identified with conventional methods. From antibiotic disc diffusion methods 74.22% ESBL strains and 9.90% MBL strains were documented. Multidrug resistant strains of *P. aeruginosa* (86/384,22.40%) were more prevalent than those of *S. aureus* (112/384,29.17%) and other bacteria in ear discharges. Imipenem and vancomicin could control to gram negative bacteria and gram positive bacteria respectively.

* Corresponding author.
E-mail address: santoshvoltaire@yahoo.co.in (S.K. Swain).
Peer review under responsibility of Chinese Medical Association.

Conclusion: Continuous and periodic evaluation of microbiological profile and antimicrobial sensitivity pattern of bacterial is essential for optimum management of CSOM patients.

Introduction

Chronic suppurative otitis media (CSOM) is chronic inflammatory condition of the middle ear cleft with permanent perforation, ear discharge and hearing loss. Clinicians dispute the duration of otorrhea for more than three months.[1,2] There are two types of CSOM and these are safe or unsafe depending on the presence of cholesteatoma[3]: Safe CSOM is CSOM without cholesteatoma and can be subdivided into *active* or *inactive* depending on whether or not infection is present. Unsafe CSOM is often associated with cholesteatoma which may lead to fatal complications.

The most common bacteria for CSOM are *Pseudomonas aeruginosa, Klebsiella* sp., *Proteus* sp., and *Staphylococcus aureus*.[4] Whereas, the bacteria commonly isolated from patients with acute otitis media (AOM) are *Moraxella catarrhalis* and *Streptococcus pneumonia, Haemophilus influenzae*.[5] Additionally, the *P. aeruginosa* had been seen as a notorious pathogen in the hospital too.[6,7] Certainly, the ability to form biofilm by these organisms contributes to their frequency in CSOM.[8] It is often found in immune compromised patient as compared to the normal patients.[9] Mostly, with several clonal variants of the bacteria were resistant to the penicillin group of antibiotics, after which different antibiotics were introduced for the control.[10] The most horrible situation is that Methicillin resistant *Staphylococcus aureus* (MRSA) strains have emerged with subsequent resistance to most commonly used antibiotics of groups, macrolides, aminoglycosides, fluoroquinolones, chloramphenicol and tetracycline and many more such as, to cephalosporin, cefepime and other betalactams, ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam and the carbapenem, imipenem. So the isolated MRSA are MDR too.[11] In a study extended spectrum beta lactamases (ESBL) and ampicillin were detected in 11 (18.3%) and 12 (20.0%) Gram negative bacteria, respectively. Whereas the MBL producer was not detected.[12]

This work describes the surveillance of bacterial flora from ear infections of patients attending the Outpatients Department (OPD) of Otorhinolaryngology department of the hospital, in a year. Antibiotic sensitivity pattern of isolated bacterial were determined to assess the variety of CSOM that would help in empirical therapy with the antimicrobial stewardship program of the teaching hospital at the eastern India. Also, the incident of ESBL and MBL strains from the CSOM were documented.

Materials and methods

Selection of CSOM patients

CSOM is a long-standing suppurative middle ear infection with permanent perforation, otorrhea and hearing loss with duration of more than three months. CSOM patients were diagnosed clinically by the consultant otolaryngologist. On the basis of clinical presentations and otoscopic examinations, patients were selected for this study. The patients of acute infections of the middle ear cleft of less than three months and traumatic perforations of the tympanic membrane were excluded from this study. The pus is collected from the ear canal in CSOM patients with the help of the sterile ear swab (Himedia mumbai PW003) by consultant otolaryngologist with all aseptic measures.

Study population

A total of 500 ear swabs from clinically diagnosed CSOM cases were collected, during July 2016 to June 2017 with sterile cotton swab sticks. Pus swabs were cultured on blood and MacConkey agar plates that were incubated at 37 °C overnight for pathogenic bacteria, which were identified according to the standard method used for bacteria (Fig. 1A—F).[13] Antibiotic susceptibility tests of isolated bacteria were done according to Clinical Laboratory Standard Institute guidelines (Fig. 2A—C), as described.[14] Standard antimicrobial disks (HiMedia, Mumbai) used for isolated bacteria were amikacin 30, gentamicin 30, ciprofloxacin, amoxyclav 30, aztreonam 30, piperacillin 100, piperacillin/tazobactam 100/10, cefepime 30, cefoperazone 75, cefoperazone/sulbactam 75/30, ceftazidime 10, ceftriaxone 10, imipenem 10, gatifloxacin 30, Oxacilin and Vancomicin. The standard MTCC strains and all the clinical isolated bacteria were subjected to antibiotic sensitivity tests with antibiotics, by the Kirby—Bauer's method (disk diffusion) detailed previously.[11]

Detection of MBL strain

The metallo β-lactamases (MBL) production was detected by the imipenem − EDTA-DDST. The organisms were considered to be MBL producers, if the increase in the inhibition zone of the β-lactam + EDTA disc was ≥5 mm (Fig. 3A—C).[15] The MBL production in this isolate, for the synergy test imipenem-EDTA disk was used developed, as followed. Earlier reports have recommended that the imipenem-EDTA disk synergy test is a reliable method for initial screening of MBL production in clinical isolates.[16]

Detection of ESBL strains

The double disc synergy test (DDST) was used to detect the ESBL producing activity of selected bacterial strains on a lawn culture on a MH agar plate. The augmentin 30 μg disc (20 μg amoxicillin and 10 μg clavulanic acid) in the middle was flanked by a disc of ceftazidime 30 μg and a disc of cefotaxime 30 μg (both third generation cephalosporin), at 30 mm apart on a lawn culture. The set up was done in triplicates
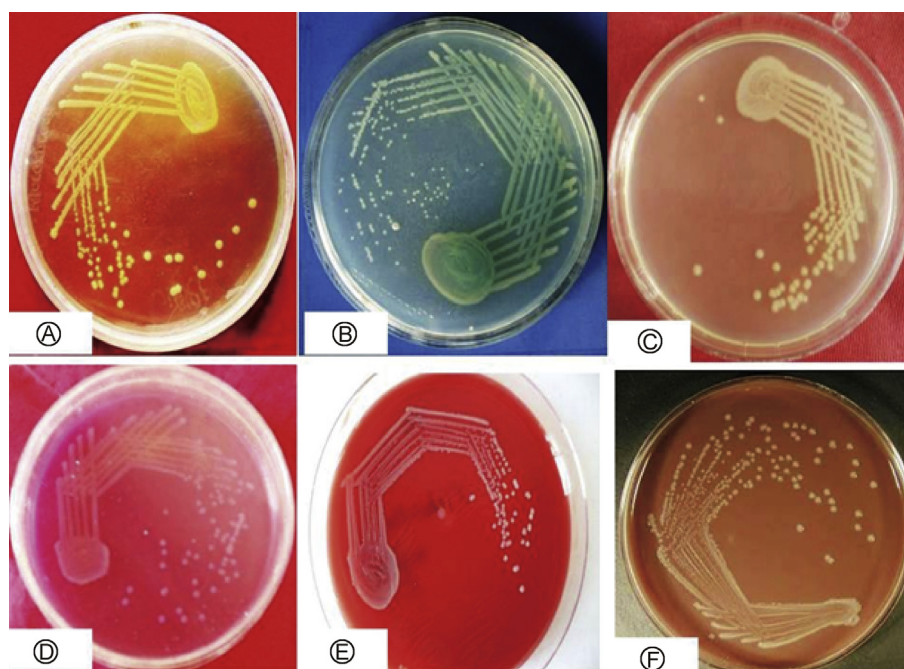
Fig. 1 Macroscopic photograph of isolated bacteria on specific medium from human chronic suppurative otitis media A: *Stphylo-coccous aureus*; B: *Pseudomonas aeruginosa*; C: *Acenetobacter baumanii*; D: *Citrobacter freundii*; E: *Enterobacter* sp.; F: *Proteus* sp.
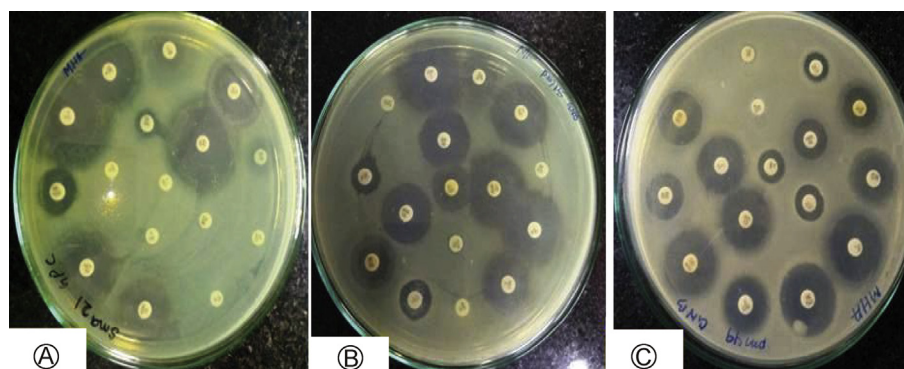

Fig. 2 Antibiotic sensitivity pattern of isolated bacteria from human chronic suppurative otitis media on MHA (Mueller Hinton Agar) A: *Psueudomonas aeruginosa*; B: *Acenetobacter baumanii*; C: *Citrobacter fruindii*.

and plates were incubated at 37 °C for overnight for observation of inhibition zones. With the augmentin disc inhibition the action of the ESBL enzyme give an inhibition zone, which was formed from a peripheral disc towards the middle, due to the synergistic action of the augmentin and the corresponding cephalosporin disc (Fig. 3D). In general, if the organism is resistant to both cephalosporins, because of the production of the ESBL enzyme, the action of augmentin deactivated the enzyme with a consequent reactivation of a cephalosporin resulting in the extension of the inhibition zone.[17]

Results

Growth of bacteria from ear swabs of CSOM patients

A total of 500 ear swabs were subjected to culture on specific culture media for bacterial growth. In this study the prevalence of bacteria among CSOM patients was 82.6%. It was observed that varieties of colonies were grown on the specific cultured medium. In certain plate single type of colony and in other cases two, three or more than 3 types of colonies were grown. The percentage of frequency of the colony was documented (Table 1). The single colony frequency was found more as compare to other types of colonies from the ear swab. Three or more colonies were treated as contaminated samples. With specific culture media different type of colonies were grown and it was identified with conventional method by both macroscopically and biochemically (Tables 2 and 3). All the grown positive bacteria were processed with catalase and coagulase and *Staphylococcus aureus* were identified basing upon the results of catalase and coagulase. Similarly all the gram negative bacterial were processed with different biochemical tests and basing up on their results the bacteria were identified (Table 3). For the species identification the carbohydrate fermentation tests were carried out and results were documented (Table 4).
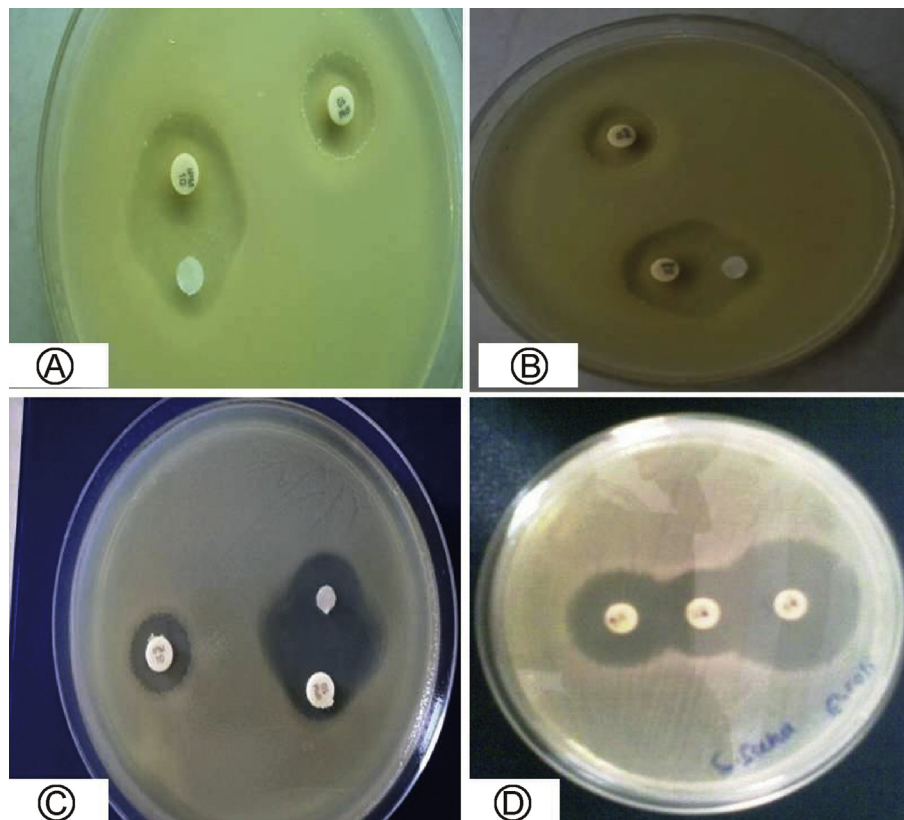
Fig. 3 Detection of Metallobetalactamases (MBL) and extended spectrum beta lactamases (ESBL) with disc diffusion methods A: MBL strain of *Pseudomonas aeruginosa*; B: MBL strain of *Klebsiela pneumaneae*; C: ESBL strain of *Citrobacter freundii*; D: ESBL strain of *Acenetobacter baumanii*.

Table 1 Number of varieties of colony observed macroscopically on the cultured plate.

No. of different Colony	Frequency	Number of bacteria	Percentage of bacteria isolated (%)
No growth	87	0	17.4
Single colony	216	216	43.2
Two colony	84	168	16.8
Three or more colony	29	Treated as contaminated	
Total	500	384	76.8

Antibiotic sensitivity test of isolated bacteria from CSOM patients

From the antibiotic tests, it was revealed that ceftazidime (CAZ) was highest resistant (78%) to *A. baumannii* and Amoxyclav is the highest sensitive (86%) to *A. baumannii*. Similarly the antibiotic resistance percentage for other bacteria was documented (Table 5). But in CSOM patients were prescribed the antibiotic amoxyclav and ciprofloxacin mainly. The ciprofloxacin is resistant to 20% to *A. baumannii*, 12% to *C. freundii*, 51% to *E. aerogenes*, 32% to *K. oxytoca*, 62% to *K. pneumonia*, 81% to *P. vulgaris*, 31% to *P. mirabilis*, 28% to *P. aeruginosa* and 43% to *S. aureus*. Similarly, Amoxyclav is resistant to 14% to *A. baumannii*, 31% to *C. freundii*, 32% to *E. aerogenes*, 16% to *K. oxytoca*, 21% to *K. pneumonia*, 25% to *P. vulgaris*, 23% to *P. mirabilis*, 17% to *P. aeruginosa* and 38% to *S. aureus* (Table 5).

Incidence of ESBL and MBL strains from CSOM patients

All the strains were screened for ESBL and MBL and it was revealed that 74.22% ESBL and 9.90% MBL strains were found. Among the ESBL strains 89 *S. aureus* and 79 *P. aeruginosa* strains were found. Similarly, 2.08% *S. aureus* and 4.43% *P. aeruginosa* MBL strains were found during this study period (Table 6).

Discussion

In this study the prevalence of bacteria among CSOM patients was 82.6%. This was in tandem with reports from other parts of 91.7%,[18] 89.4%,[19] 89.5%,[20] 100%[21] and Nigeria, 81.9%.[22] Gram-negative bacteria, 59.6% were the dominant isolates of the discharging ears compared to gram-positive

Table 2 dentification of bacteria with culture morphology.

Bacterium	MTCC no.	Agar media	Colony morphology
Staphylococcus aureus	7443	Nutrient agar	Golden yellow, opaque, circular colonies white butyrous consistency
		Mannitol salt agar	Yellow colonies,
Acinetobacter baumannii	1425	Nutrient agar (NA)	Colourless smooth, opaque, raised and pinpoint colonies.
		MacConkey agar	Colourless smooth, opaque, raised, NLF colonies
Enterobacter aerogenes	2990	Blood agar	Small, round and pin -point colony.
		MacConkey agar	LF and mucoid colonies
Citrobacter freundii	1658	MacConkey agar	Late LF colonies light pink after 48 h
Klebsiella sp.	4031	MacConkey agar	LF, pink, mucoid colonies.
		CLED agar	Yellow and mucoid colonies
Proteus sp.	1771	Blood agar	Swarming colonies
		CLED	translucent blue colonies
Pseudomonas aeruginosa	1688	Nutrient agar,	Large, irregular opaque colonies, with bluish green pigment.

MTCC: Microbial type culture collection; Na: not available; EMB: Eosin methylene blue agar; XLD: Xylose lysine deoxycholate; CLED: cystine lactose electrolyte deficient medium; LF, lactose fermenting; NLF: Non-lactose fermenting.

Table 3 Summary of results of biochemical tests of isolated Gram-negative bacteria.

Bacteria	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate	Motility
A. baumannii	+	−	−	−	+	+	V	ND	−	M
Citrobacter sp.	+	−	−	+	−	+	−	K/A + H_2S	+	M
Enterobacter sp	+	−	−	−	+	+	V	A/A	+	M
K. oxytoca	+	−	+	−	+	+	+	A/AG	+	NM
K. pneumoniae	+	−	−	−	+	+	+	A/AG	+	NM
P. vulgaris	+	−	−	+	−	V	+	K/A H_2S	+	M
P. mirabilis	+	−	+	+	−	V	+	K/A H_2S	+	M
P. aeruginosa	+	+	−	−	−	+	+	ND	+	M

+: positive; −: negative; V: variable; MR: methyl red; VP: Voges-Proskauer; TSI: triple sugar iron; A: acid; K: alkali; G: gas; H_2S: H_2S production; M: motile; NM: non-motile; ND: not done.

Table 4 Summary of results of carbohydrate fermentation tests of isolated Gram-negative bacteria.

Bacteria	Glucose	Lactose	Sucrose	Maltose	Mannitol
A. baumannii	−	−	−	−	−
Citrobacter sp.	A + G	LLF	+	+	+
Enterobacter sp.	A + G	A	+	+	+
P. vulgaris	G	−	−	−	+
P. mirabilis	G	−	−	−	+
K. oxytoca	A + G	A	+	−	+
K. pneumoniae	A + G	A	+	−	+
P. aeruginosa	A	−	+	+	V

A: acid; A + G: acid + gas; V: variable; LLF: late lactose fermentation; LSF: late sucrose fermentation; +: positive; −: negative.

bacteria. Similar reports were seen from Gonder 56.4%,[20] Dessie 74.2%,[18] Addis Ababa 60.5%[23] and Nigeria 75%[24] though the proportion varies.

The CSOM is a major health problem both in children and adults world-wide, but more so in developing countries. It can cause chronic hearing loss which has a negative impact on the development of speech, language and social inter-action as well as school and workplace performance and is responsible for significant morbidity and mortality due to complications. According to a report by WHO, India belongs to the highest (>4%) CSOM prevalent countries.[4] Topical antibiotics are the mainstay of therapy while systemic anti-biotics are given in acute exacerbations and in complications due to CSOM.[25] The poor living conditions, less access to medical care, poor medical treatment, recurrent upper respiratory tract infections and the nasal diseases have been recognized as risk factors for CSOM.[25] Atticoantral disease is the most commonly involved with the posterior superior part of the middle ear cleft and it is characterized by the for-mation of a retraction pocket with cholesteatoma; eventu-ally it is considered to be a dangerous form of the disease because of the development of several intracranial and extra-cranial complications.[26] 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 in community too.[27] Although the clinical relevance of Coagulase negative *Staphylococcus Sp.* (CONS) is still controversial; patients at risk of CONS in-fections include neonates, those with intravascular cathe-ters, prosthetic devices and surgical wounds in the 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.[28,29] Particularly, several

Table 5   Percentage of antibiotic resistance with corresponding bacteria (μg/disc).

Bacteria	AK	GEN	CIP	AMC	AT	PI	PIT	CPM	CPZ	CFS	CAZ	CTR	IPM	GAT	OX	V
A. baumannii	23	30	20	14	71	30	21	71	62	34	78	72	21	20	—	—
C. freundii	7	8	12	31	63	40	39	69	52	15	82	74	31	22	—	—
E. aerogenes	78	56	51	32	60	78	34	61	56	24	74	68	42	16	—	—
K. oxytoca	24	36	32	16	74	82	25	54	62	21	82	74	38	19	—	—
K. pneumoniae	63	83	62	21	61	87	28	57	71	21	89	82	56	71	—	—
P. vulgaris	69	48	81	25	58	91	34	64	52	16	91	92	58	69	—	—
P. mirabilis	49	54	31	23	46	69	21	78	61	14	78	79	37	78	—	—
P. aeruginosa	68	59	28	17	51	67	23	83	73	31	73	74	39	31	—	—
S.aureus	75	46	43	38	87	59	—	68	53	48	27	43	—	39	54	36

Ak: amikacin 30; GEN: gentamicin 30; CIP: ciprofloxacin; AMC: amoxyclav 30; AT: aztreonam 30; PI: piperacillin 100; PIT: piperacillin/tazobactam 100/10; CPM: cefepime 30; CPZ: cefoperazone 75; CFS: cefoperazone/sulbactam 75/30; CAZ: ceftazidime 10; CTR: ceftriaxone 10; IPM: imipenem 10 (Carbap., carbapenem); GAT: gatifloxacin 30; OX: Oxacilin; V:Vancomicin.

Table 6   Number of isolated ESBL and MBL strains from Gram negative (GN) bacteria.

Bacteria	Number of isolated strains	%	ESBL strain	% with respective bacteria	% with total bacteria	MBL strain	% with respective bacteria	% with total bacteria
A. baumannii	53	13.80	34	64.15	8.85	6	11.32	1.56
C. freundii	26	6.77	11	42.31	2.86	1	3.85	0.26
E. aerogenes	32	8.33	18	56.25	4.69	0	0	0.00
K. oxytoca	24	6.25	20	83.33	5.21	0	0	0.00
K. pneumoniae	18	4.69	9	50	2.34	1	5.56	0.26
P. vulgaris	23	5.99	21	91.3	5.47	2	8.70	0.52
P. mirabilis	10	2.60	4	40	1.04	3	30.00	0.78
P. aeruginosa	112	29.17	89	79.46	23.18	8	7.14	2.08
S. aureus	86	22.40	79	91.86	20.57	17	19.77	4.43
Total	384	100.00	285	74.22	74.22	38	9.90	9.90

ESBL: Extended spectrum beta lactamse; MBL: mettalo beta lactamase.

clonal variants of *S. aureus* and MRSA were reported 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%).[30]

In our study, from 500 samples 384 bacteria were isolated. In other studies, Nazir et al[31] and Sanjana et al[32] have reported less number of bacteria as compare to our study results. Gram negative bacteria predominance (60.6%) matches other studies in India.[33] *S. aureus* was the predominant bacteria followed by *P. aeruginosa* which are in opposite with other studies.[4,34] Whereas similar results reported in other studies i.e. *S. aureus* as predominant isolate followed by *P. aeruginosa*.[35—37] In this study higher resistance demonstrated in Gram positive bacteria 54% *S. aureus* exhibiting methicillin resistance. On the other hand resistance among Gram negative bacteria was much lower with 9.90% MBL producer detected but high rate of 74.22% ESBL strains documented.

Conclusion

CSOM is a common clinical entity where topical and systemic antibiotic are the main treatment. However the emergence of antibiotic resistant strains is leading to increasing treatment failure. MDR strains of *P. aeruginosa* and MRSA were most prevalent in ear discharges of patients with CSOM. Continuous and periodic evaluation of microbiological profile and antimicrobial sensitivity pattern of bacterial is essential for optimum management of CSOM patients.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

References

1. Arslan IB, Genc S, Kayhan BC, Gumussoy M, Ozel G, Cukurova I. Bacterial change in external auditory canal upon antisepsis with povidone-iodine during tympanoplasty. *Eur Arch Otorhinolaryngol*. 2015;272:551—555.

2. Thompson PL. *Effect of Antibiotics for Otitis Media on Mastoiditis in Children*. London: University of London, School of Pharmacy; 2009.

3. Chirwa M, Mulwafu W, Aswani JM, Masinde PW, Mkakosya R, Soko D. Microbiology of chronic suppurative otitis media at Queen Elizabeth Central Hospital, Blantyre, Malawi: a cross-sectional descriptive study. *Malawi Med J*. 2015;27:120—124.

4. Shyamala R, Reddy PS. The study of bacteriological agents of chronic suppurative otitis media-Aerobic culture and evaluation. *J Microbiol Biotechnol Res*. 2017;2:152—162.

5. Berman S. Otitis media in children. *N Engl J Med*. 1995;332:1560—1565.

6. Sahu MC, Dubey D, Rath S, Debata NK, Padhy RN. Multidrug resistance of Pseudomonas aeruginosa as known from surveillance of nosocomial and community infections in an Indian teaching hospital. *J Publ Health*. 2012;20:413—423.

7. Afolabi OA, Fadare JO, Omokanye HK, et al. Socioeconomic challenges of chronic suppurative otitis media management in state tertiary health facility in Nigeria. *Egypt J Ear Nose Throat Allied Sci*. 2014;15:17—22.

8. Couzos S, Lea T, Mueller R, Murray R, Culbong M. Effectiveness of ototopical antibiotics for chronic suppurative otitis media in Aboriginal children: a community-based, multicentre, double-blind randomised controlled trial. *Med J Aust*. 2003;179:185—190.

9. Bluestone CD, Klein JO. In: *Otitis Media in Infants and Children*. 3rd ed. Philadelphia, PA: WB Saunders; 2001:79—101.

10. Sahu MC, Padhy RN. Bayesian evaluation of two conventional diagnostic methods for pathogenic fungal infections. *J Acute Med*. 2014;4:109—119.

11. Askarian M, Hosseini RS, Kheirandish P, Assadian O. Incidence and outcome of nosocomial infections in female burn patients in Shiraz, Iran. *Am J Infect Control*. 2004;32:23—26.

12. Khatoon A, Rizvi M, Sultan A, et al. Chronic suppurative otitis media: a clinico-microbiological menace. *Int J Res Med Sci*. 2015;3:1932—1936.

13. Ahmed B, Hydri AS, Ejaz A, Farooq S, Zaidi SK, Afridi AA. Microbiology of ear discharge in Quetta. *J Coll Phys Surg Pak*. 2005;15:583—584.

14. 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—317.

15. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of pseudomonas aeruginosa producing metallo-beta-lactamases in a large centralized laboratory. *J Clin Microbiol*. 2005;43:3129—3135.

16. Irfan S, Zafar A, Guhar D, Ahsan T, Hasan R. Metallo-beta-lactamase-producing clinical isolates of Acinetobacter species and Pseudomonas aeruginosa from intensive care unit patients of a tertiary care hospital. *Indian J Med Microbiol*. 2008;26:243—245.

17. Peña C, Pujol M, Ricart A, et al. Risk factors for faecal carriage of Klebsiella pneumoniae producing extended spectrum beta-lactamase (ESBL-KP) in the intensive care unit. *J Hosp Infect*. 1997;35:9—16.

18. Abera B, Kibret M. Bacteriology and antimicrobial susceptibility of otitis media at dessie regional health research laboratory, Ethiopia. *Ethiopian J Health Develop*. 2011;25:161—167.

19. Seid A, Deribe F, Ali K, Kibru G. Bacterial otitis media in all age group of patients seen at Dessie referral hospital, North East Ethiopia. *Egypt J Ear Nose Throat Allied Sci*. 2013;14:73—78.

20. Muluye D, Wondimeneh Y, Ferede G, Moges F, Nega T. Bacterial isolates and drug susceptibility patterns of ear discharge from patients with ear infection at Gondar University Hospital, Northwest Ethiopia. *BMC Ear Nose Throat Disord*. 2013;13:10.

21. Diriba M, Solomon G, Hailu N. Isolation and antimicrobial susceptibility pattern of bacterial pathogens causing otitis media in children in Jimma hospital, South Western Ethiopia. *Ethiop J Health Sci*. 2004;14:89—100.

22. Osazuwa F, Osazuwa E, Osime C, et al. Etiologic agents of otitis media in Benin city, Nigeria. *N Am J Med Sci*. 2011;3:95—98.

23. Ferede D, Geyid A, Lulseged S, et al. Drug susceptibility pattern of bacterial isolates from children with chronic suppurative otitis media. *Ethiop J Health Dev*. 2001;15:89—96.

24. Iseh KR, Adegbite T. Pattern and bacteriology of acute suppurative otitis media in Sokoto, Nigeria. *Ann Afri Med*. 2004;3:164—166.

25. Prajna L, Vijayakumar A. *Atlas of Fungal Corneal Ulcers Clinical Features and Laboratory Identification Methods*. New Delhi: Jaypee Broth Med Publishers; 2008:24—28.

26. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. *PA Wayne*. 2011;1:100—181.

27. Dash A, Sahu K, Senapati JN, et al. Surveillance of antibiotic sensitivity and resistance pattern of bacteria isolated from orthopaedic wound discharge. *Int J Pharm Sci Rev Res*. 2016;36:208—211.

28. Vikram BK, Khaja N, Udayashankar SG, Venkatesha BK, Manjunath D. Clinico-epidemiological study of complicated and uncomplicated chronic suppurative otitis media. *J Laryngol Otol*. 2008;122:442—446.

29. Chowdhury MA, Alauddin M. Comparative study between tubotympanic and atticoantral types of chronic suppurative otitis media. *Bangladesh Med Res Counc Bull*. 2002;28:36—44.

30. Zong Z, Peng C, Lü X. Diversity of SCCmec elements in methicillin-resistant coagulase-negative staphylococci clinical isolates. *PLoS One*. 2011;6:e20191.

31. Sanjana RK, Singh YI, Reddy NS. Aerobic bacteriology of chronic suppurative otitis media (CSOM) in a tertiary care hospital: a retrospective study. *J Coll Med Sci Nepal*. 2012;7:1—8.

32. Kumar H, Seth S. Bacterial and fungal study of chronic suppurative otitis media. *J Clin Diagn Res*. 2011;5:1224—1227.

33. Kumar KR, Navya S, Basavarajappa KG. A study of bacterial profile and antibiotic susceptibility pattern of chronic suppurative otitis media among patients attending a tertiary care centre, davangere. *Sch J App Med Sci*. 2014;2:1606—1612.

34. Sharma V, Kaur G. Microbiology and antimicrobial susceptibility pattern of cases of chronic suppurative otitis media in a tertiary care teaching hospital. *Int J Bioassays*. 2014;3:3033—3035.

35. Prakash M, Lakshmi K, Anuradha S, Swathi GN. Bacteriological profile and their antibiotic susceptibility pattern of cases of chronic suppurative otitis media. *Asian J Pharm Clin Res*. 2013;6:210—212.

36. Mozafari NK, Sepehri G, Khatmi H, Shakibaie MR. Isolation and antimicrobial susceptibility of bacteria from chronic suppurative otitis media patients in kerman, Iran. *Iran Red Crescent Med J*. 2011;13:891—894.

37. Ahmed K, Mir A, Jan M, Imran R, Shah G, Latif A. Prevalence of bacteria in chronic suppurative otitis media patients and their sensitivity patterns against various antibiotics in human population of gilgit. *Pakistan J Zool*. 2013;45:1647—1653.

Edited by Yu-Xin Fang