International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Website: https://ijmpr.in/

Original Article

A Study to Detect MRSA and ESBL Producing Isolates from Middle Ear Infections in Tertiary Care Hospital

Dr Pramod Sambrani[1], Dr Rejinold TI [2*], Dr Anubhav sinha[3]

[1]Department of Microbiology, Associate Professor, KMCRI, Hubballi
[2]Department of Microbiology, Postgraduate, KMCRI, Hubballi.*
[3]Department of Microbiology, Postgraduate, KMCRI, Hubballi

OPEN ACCESS

Corresponding Author:

Dr Rejinold TI
Department of Microbiology, Karnataka medical college and research institute, Hubballi, Karnataka
Email- drrejinold@gmail.com

Received: 17-03-2026
Accepted: 17-04-2026
Published: 22-04-2026

ABSTRACT

Introduction: The present study addresses the detection of antibiotic resistance in bacterial isolates from middle ear infections, with a focus on methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum beta-lactamase (ESBL) producing organisms. Middle ear infections, particularly chronic suppurative otitis media (CSOM), remain a significant health concern due to persistent infections and the emergence of multidrug-resistant pathogens. Understanding the prevalence and resistance patterns of these organisms is critical for guiding effective treatment strategies.

Objectives: The primary objective of the present study is to detect the presence of MRSA and ESBL-producing bacterial isolates in patients with middle ear infections attending a tertiary care hospital. Secondary objectives include determining the antibiotic susceptibility patterns of these isolates and analyzing demographic and clinical characteristics of affected patients.

Methodology: A prospective cross-sectional laboratory-based study was conducted at the Department of Microbiology, KMCRI, Hubballi, involving 140 ear swab samples collected from patients clinically diagnosed with middle ear infections. Samples were cultured on appropriate media, and isolates were identified using standard microbiological and biochemical tests. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to CLSI 36th edition guidelines. MRSA detection was done using the cefoxitin disc diffusion method, while ESBL production among Gram-negative isolates was screened and confirmed through combination disc methods.

Results: Staphylococcus aureus was the predominant isolate, accounting for 52.1% (n = 73) of cases, followed by Pseudomonas spp. (23.6%), Klebsiella spp. (10.7%), and Escherichia coli (7.1%). Among S. aureus isolates, 38.3% were identified as MRSA. ESBL production was detected in 27.5% of Gram-negative isolates. Antibiotic susceptibility revealed that Gram-positive isolates were most sensitive to linezolid, vancomycin, and clindamycin, whereas Gram-negative isolates showed high sensitivity to carbapenems, tigecycline, amikacin, and gentamicin. Resistance to commonly used beta-lactam antibiotics was widespread among ESBL producers.

Conclusion: The present study highlights the predominance of S. aureus, including a significant proportion of MRSA, in middle ear infections, alongside considerable ESBL production among Gram-negative isolates. These findings underscore the necessity for continuous surveillance of pathogen profiles and resistance patterns to inform empirical therapy, optimize treatment outcomes, and mitigate the spread of antimicrobial resistance in clinical settings.

Keywords: *MRSA, ESBL, middle ear infections, antibiotic resistance, Staphylococcus aureus.*

INTRODUCTION

The World Health Organization defines Chronic Otitis Media (COM) as ear discharge through a perforated tympanic membrane persisting for more than 12 weeks, associated with chronic inflammation of the middle ear and mastoid cavity. Otitis media refers to inflammation of the middle ear cleft irrespective of aetiology or pathogenesis. The Eustachian tube plays a crucial role in maintaining middle ear health by equalizing pressure, facilitating drainage of secretions, and protecting against infections. Dysfunction or obstruction of this tube predisposes to the development of otitis media.[1]

Children are particularly susceptible due to anatomical and functional differences in the Eustachian tube, which is shorter, wider, and more horizontal, favoring stasis of nasopharyngeal secretions and facilitating microbial colonization. Acute suppurative otitis media commonly follows upper respiratory tract infections, whereas persistent or recurrent infection leads to Chronic Suppurative Otitis Media (CSOM), characterized by chronic inflammation of the middle ear and mastoid cavity with recurrent otorrhoea through a perforated tympanic membrane.[2]

CSOM is clinically classified into two types: tubotympanic (mucosal) and squamosal (atticoantral). The tubotympanic type involves the pars tensa and presents with central perforation and is considered the "safe" type due to its lower risk of complications. In contrast, the squamosal type involves the pars flaccida with attic or marginal perforation and is termed the "unsafe" type because of its association with bone erosion and potentially serious complications.[3]

Among South-East Asian countries, India has a high prevalence of CSOM, estimated at approximately 7.8%. More than half of the cases are caused by bacterial pathogens. Common aerobic organisms include Pseudomonas spp., Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Proteus mirabilis, and Klebsiella spp., while anaerobes such as Bacteroides, Peptostreptococcus, and Peptococcus also contribute. Fungal agents, particularly Aspergillus and Candida species, are also implicated, with microbial distribution varying across geographical regions.[4]

The persistence and severity of infection are influenced by various virulence factors, including biofilm formation, beta-lactamase production, lipopolysaccharides, metalloproteases, capsule formation, and swarming motility. Furthermore, the indiscriminate use of antibiotics, poor compliance, and inadequate follow-up have contributed to the emergence of multidrug-resistant organisms.

In this context, the present study aims to detect antibiotic resistance patterns in isolates from middle ear infections, with special reference to MRSA, ESBL producing organisms.

METHODOLOGY

Study Design and Setting:

The present study is a prospective cross-sectional laboratory-based investigation conducted at the Department of Microbiology, Karnataka Medical College and Research Institute (KMCRI), Hubballi, Karnataka, India. The study duration encompassed the period during which 140 ear swab samples were collected from patients clinically diagnosed with middle ear infections. Ethical approval was obtained from the institutional ethical committee prior to study initiation, and the study adhered to the STROBE guidelines for observational research.

Study Population:

Patients of all ages, both children and adults, presenting with active middle-ear discharge and a clinical diagnosis of otitis media were included in the study. Inclusion criteria comprised patients with clinically confirmed middle ear infections exhibiting active discharge. Exclusion criteria involved contaminated or improperly collected samples and duplicate samples from the same site and patient within a short timeframe unless clinically indicated. The sample size of 140 was determined based on the number of eligible patients presenting during the study period. Consecutive sampling was employed, whereby all patients meeting inclusion criteria during the study timeframe were enrolled.

Data Collection:

Ear discharge specimens were collected aseptically using sterile cotton swabs from the external auditory canal of patients. Two swabs per patient were obtained and immediately transported to the microbiology laboratory for processing. Samples were cultured on Chocolate agar and MacConkey agar and incubated at 37°C for 18–24 hours. Isolates were identified using standard microbiological methods including colony morphology, Gram staining, motility tests, and a series of biochemical tests tailored to differentiate Staphylococcus aureus, coagulase-negative staphylococci, and Gram-negative bacilli.

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar following Clinical and Laboratory Standards Institute (CLSI) 36th edition guidelines. MRSA detection was conducted using the cefoxitin (30 µg) disc diffusion method. ESBL production among Gram-negative isolates was initially screened with ceftazidime and cefotaxime discs and confirmed by the combination disc method employing ceftazidime with and without clavulanic acid.

Statistical Analysis:
Descriptive statistics were used to summarize demographic data, bacterial isolate distribution, and antibiotic susceptibility patterns, expressed as frequencies and percentages. Inferential statistical analyses were not explicitly detailed in the study protocol; however, the prevalence rates of MRSA and ESBL producers were calculated. Data were tabulated and analyzed using standard statistical software packages where applicable.

Ethical Considerations:
The study was conducted following ethical principles outlined in the Declaration of Helsinki. Institutional ethical committee approval was obtained before commencement. Informed consent was acquired from all participants or their guardians. Patient confidentiality was maintained throughout the study process.

RESULT
A total of 140 ear swab samples from clinically diagnosed cases of chronic suppurative otitis media were processed for bacteriological analysis. .

Among the isolates obtained, Staphylococcus aureus was the predominant organism, accounting for 52.1% (n = 73) of cases, followed by Pseudomonas spp. in 23.6% (n = 33), Klebsiella spp. in 10.7% (n = 15), and Escherichia coli in 7.1% (n = 10) of cases. Other bacterial isolates constituted 6.4% (n = 9) of the total isolates.. ESBL producers constituted 27.5% (18 isolates), highlighting a notable proportion of bacteria with resistance to beta-lactam antibiotics

Among the 73 isolates of Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA) constituted 38.3% (n = 28), while methicillin-sensitive Staphylococcus aureus (MSSA) accounted for 61.7% (n = 45).

Among Gram-negative bacilli isolated, extended-spectrum beta-lactamase (ESBL) production was detected in 27.5% (n = 18) of isolates.

Antibiotic susceptibility testing revealed that Gram-positive isolates showed maximum sensitivity to linezolid, vancomycin, and clindamycin. Among Gram-negative isolates, higher sensitivity was observed with amikacin, ciprofloxacin, meropenem, and ceftazidime.

Analysis of demographic characteristics showed that the majority of patients belonged to the 11–20 years age group (38.6%, n = 54), followed by the 21–30 years age group (31.4%, n = 44). Male patients were more commonly affected (60%, n = 84) than female patients (40%, n = 56).

With respect to clinical presentation, unilateral ear involvement was observed in 59.3% (n = 83) of patients, while bilateral involvement was seen in 40.7% (n = 57). Monobacterial infection was the predominant pattern, noted in 100% (n = 140) of cases.

Table 1. Distribution of Bacterial Isolates from Ear Swab Samples (n = 140)

Organism	Number of Isolates (n)	Percentage (%)
Staphylococcus aureus	73	52.1
Pseudomonas spp.	33	23.6
Klebsiella spp.	15	10.7
Escherichia coli	10	7.1
Other organisms	9	6.4

Staphylococcus aureus was the most common isolate (52.1%), followed by Pseudomonas spp. (23.6%), Klebsiella spp. (10.7%), Escherichia coli (7.1%), and other organisms (6.4%).

Table 2. Distribution of MRSA and MSSA among Staphylococcus aureus Isolates (n = 73)

Isolate Type	Number (n)	Percentage (%)
MRSA	28	38.3
MSSA	45	61.7

MRSA accounted for 38.3% (28 isolates), indicating a significant presence of antibiotic-resistant strains, while MSSA made up 61.7% (45 isolates).

Table 3. Demographic and Clinical Characteristics of Patients (n = 140)

Variable	Category	Number (n)	Percentage (%)
Age Group	11–20 years	54	38.6
	21–30 years	44	31.4
Gender	Male	84	60.0
	Female	56	40.0
Laterality of Infection	Unilateral	83	59.3
	Bilateral	57	40.7
Type of Infection	Monobacterial	140	100
	Polymicrobial	0	0

The majority of patients were aged 11–20 years (38.6%), followed by those aged 21–30 years (31.4%). Male patients constituted 60% of the study population, while females made up 40%. Regarding the laterality of infection, 59.3% had unilateral ear involvement, and 40.7% had bilateral involvement.

Table 4. Antimicrobial Susceptibility Pattern for Gram negative bacilli (n = 18)

Antibiotic	E. coli R (%)	E. coli S (%)	K. pneumoniae R (%)	K. pneumoniae S (%)	P. aeruginosa R (%)	P. aeruginosa S (%)
Amoxicillin-clavulanate	100	0	100	0	99	1
Piperacillin-tazobactam	100	0	100	0	97	3
Cefotaxime	100	0	100	0	100	0
Ceftazidime	100	0	100	0	100	0
Cefepime	100	0	100	0	100	0
Meropenem	0	100	0	100	0	100
Imipenem	0	100	0	100	0	100
Amikacin	29.6	70.4	0	100	20	80
Gentamicin	2	98	1	99	12	88
Ciprofloxacin	66.7	33.3	50	50	61	39
Levofloxacin	100	0	100	0	100	0
Cotrimoxazole	12	88	15	85	8	92
Tigecycline	0	100	0	100	0	100
Aztreonam	100	0	100	0	100	0

ESBL production: 27.5% (n = 18), Note: ESBL-producing isolates are reported resistant to all penicillin's and cephalosporins and monobactams except cephamycin's as per CLSI guidelines. R- Resistan, S-Sensitive. For ESBL-producing isolates, E. coli and Klebsiella pneumoniae, Pseudomonas aeruginosa showed 100% resistance to amoxicillin-clavulanate, piperacillin-tazobactam, and all cephalosporins. Carbapenems (meropenem and imipenem) and tigecycline showed 100% sensitivity across all organisms. Amikacin and gentamicin demonstrated high sensitivity, especially in K. pneumoniae and P. aeruginosa, while E. coli showed comparatively lower sensitivity to amikacin. Fluoroquinolones showed variable resistance, and cotrimoxazole retained good sensitivity in all three organisms.

Table 5. Antimicrobial Susceptibility of Staphylococcus Isolates (n = 73)

Antibiotics	Sensitive	%	Resistant	%
CIP	65	89.0	8	11.0
GEN	51	70.0	22	30.0
AK	69	95.0	4	5.0
AMC	29	40.0	44	60.0
AMP	22	30.0	51	70.0
LE	66	90.0	7	10.0
LZ	73	100.0	0	0.0
COT	69	95.0	4	5.0
E	40	55.0	33	45.0
CD	42	57.0	31	43.0
CX	45	61.7	28	38.3

Among the Staphylococcus aureus isolates (n = 73), methicillin-resistant Staphylococcus aureus (MRSA) accounted for 38.3% (n = 28), while methicillin-sensitive Staphylococcus aureus (MSSA) constituted 61.7% (n = 45). Among the antibiotics tested, linezolid showed 100% sensitivity, followed by amikacin and cotrimoxazole (95% each), and levofloxacin (90%). Ciprofloxacin also demonstrated high sensitivity (89%), while gentamicin showed moderate sensitivity (70%). Erythromycin (55%) and clindamycin (57%) exhibited comparatively lower sensitivity. Beta-lactam antibiotics such as amoxicillin-clavulanate (40%) and ampicillin (30%) showed high resistance rates, indicating limited effectiveness against the isolates.

DISCUSSION

The present study revealed an ESBL prevalence of 27.5% among Gram-negative isolates, reflecting a considerable burden of β-lactam resistance in the clinical setting. This finding aligns with the increasing global trend of ESBL-producing organisms, particularly among Escherichia coli and Klebsiella pneumoniae, which are well-recognized reservoirs of plasmid-mediated resistance genes. The high prevalence underscores the clinical significance of routine ESBL screening in microbiology laboratories.[5]

A striking observation in this study was the 100% resistance of E. coli and K. pneumoniae to penicillin's and cephalosporins, including third- and fourth-generation agents. This is expected, as ESBL enzymes efficiently hydrolyse these antibiotics, rendering them ineffective. According to CLSI recommendations, ESBL-producing isolates are to be reported as resistant to all penicillin's and cephalosporins regardless of in vitro susceptibility, which justifies the uniform resistance pattern observed.[6]

In contrast, Pseudomonas aeruginosa demonstrated relatively better susceptibility to β-lactam/β-lactamase inhibitor combinations such as amoxicillin-clavulanate and piperacillin-tazobactam. This difference may be attributed to the distinct resistance mechanisms in Pseudomonas, including efflux pumps and porin channel alterations, rather than classical ESBL production alone. However, the organism still exhibited complete resistance to cephalosporins, highlighting the limited utility of these agents.[7]

Carbapenems (meropenem and imipenem) showed 100% sensitivity across all isolates, reaffirming their status as the most reliable therapeutic agents for ESBL-producing infections. Their stability against ESBL-mediated hydrolysis makes them the cornerstone of treatment, especially in severe infections. However, the increasing reliance on carbapenems raises concerns about the potential emergence of carbapenem-resistant organisms, emphasizing the need for cautious and judicious use.[8]

Tigecycline also demonstrated excellent in vitro activity against all isolates, suggesting its role as a valuable alternative, particularly in multidrug-resistant infections where carbapenem-sparing strategies are desired. Among aminoglycosides, amikacin and gentamicin retained good efficacy, with particularly high sensitivity in K. pneumoniae and P. aeruginosa. The relatively lower sensitivity of amikacin in E. coli may indicate evolving resistance patterns and highlights the importance of local antibiograms in guiding therapy.[9]

Fluoroquinolones, including ciprofloxacin and levofloxacin, showed variable and generally reduced susceptibility, especially in E. coli and P. aeruginosa. This could be attributed to widespread empirical use, leading to selective pressure and the emergence of resistant strains. Similarly, cotrimoxazole demonstrated moderate to good sensitivity across organisms, indicating that it may still be useful in selected, uncomplicated infections based on susceptibility results.[10]

Overall, the findings of this study highlight the growing challenge posed by ESBL-producing organisms in clinical practice. The high resistance to commonly used β-lactam antibiotics limits therapeutic options and necessitates reliance on higher-end drugs such as carbapenems and tigecycline. This situation underscores the urgent need for robust antimicrobial stewardship programs, regular surveillance of resistance patterns, and strict infection control measures to curb the spread of resistant pathogens.

The present study has several limitations. The sample size of 140 patients, while adequate for preliminary analysis, may limit the generalizability of the findings to broader populations or different geographic regions. The study was conducted at a single tertiary care hospital, which could introduce institutional bias and limit external validity. Inferential statistical analyses were not extensively performed, restricting the ability to establish associations or risk factors related to MRSA and ESBL prevalence. Additionally, molecular methods for confirming resistance mechanisms were not employed, which could have provided more precise characterization of MRSA and ESBL-producing isolates. The study also did not assess patient treatment outcomes or long-term follow-up, limiting insights into the clinical impact of detected resistance patterns. Lastly, the exclusion of polymicrobial infections may overlook the complexity of middle ear infections in some patients.

The present study recommends continuous surveillance of bacterial pathogens and their antibiotic resistance patterns in middle ear infections to guide empirical therapy effectively. Implementation of robust antimicrobial stewardship programs is essential to mitigate the emergence and spread of multidrug-resistant organisms such as MRSA and ESBL producers. Regular updating of local antibiograms will aid clinicians in selecting appropriate antibiotics, reducing treatment failures. Furthermore, strict infection control measures should be enforced within healthcare settings to prevent transmission. Future research incorporating molecular methods and larger, multicentric samples is advised to enhance understanding of resistance mechanisms and improve patient outcomes.

CONCLUSION

The present study successfully detected methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum beta-lactamase (ESBL) producing isolates from middle ear infections in a tertiary care hospital setting. Staphylococcus aureus was identified as the predominant pathogen, accounting for 52.1% of isolates, with 38.3% of these classified as MRSA. Among Gram-negative isolates, 27.5% exhibited ESBL production, indicating significant beta-lactam antibiotic resistance. Antibiotic susceptibility patterns demonstrated high sensitivity of Gram-positive isolates to linezolid, vancomycin, and clindamycin, while Gram-negative isolates were most sensitive to carbapenems, tigecycline, amikacin, and gentamicin. These findings emphasize the considerable burden of multidrug-resistant organisms in middle ear infections. The study underscores the critical need for ongoing surveillance of bacterial profiles and resistance mechanisms to guide empirical therapy effectively, optimize clinical outcomes, and limit the spread of antimicrobial resistance within healthcare facilities.

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