RESEARCH ARTICLE

# Antibiotic susceptibility pattern of bacteria in patients with middle ear infection

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## Abstract

**Objective:** To assess the antibiotic resistance profiles of bacterial pathogens isolated from patients with middle ear infections.

**Method: T**he cross-sectional study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan, from November 2022 to December 2023, and comprised swab samples from middle ear infections collected from the Bolan Medical Complex Hospital, Quetta. The samples were promptly cultured, and positive cultures underwent biochemical tests, antibiotic susceptibility testing, and polymerase chain reaction analysis. Data was analysed using SPSS 22.

**Results:** Of 1,000 samples, 788 (78.8%) tested positive for pathogenic bacteria; 404 (40.4%) from male patients, 384 (38.4%) from female patients, and 474 (47.4%) from patients aged 1-15 years. Pseudomonas aeruginosa was the most prevalent bacterium 192 (24.3%), followed by staphylococcus aureus 154 (19.5%), escherichia coli 99 (12.5%), klebsiella pneumonia 107 (13.5%) and clostridium perfringens 71 (9%). Demographic analysis revealed a higher incidence among males 404 (40.4%) compared to females 384 (38.4%), with illiterate individuals being significantly more affected 568 (56.8%) than literate individuals 220 (22%). Socioeconomic factors also influenced infection rates, with more cases in those having a lower class 544 (54.4%). Infections were observed in all age groups, with the highest prevalence in individuals aged 1-15 years 474 (47.4%). Polymerase chain reaction analysis confirmed the bacterial presence, identifying specific gene bands for each organism. All isolates showed high resistance to metronidazole, tetracycline, lincomycin, and penicillin G.

**Conclusion:** Middle ear infections were most prevalent in patients aged 1-15 years. The bacterial isolates demonstrated multidrug resistance, highlighting the importance of enhancing efforts to isolate microorganisms and determine their susceptibility patterns in order to improve the treatment outcomes for otitis media.


**Keywords:** Antibiotic resistance, Bacterial profiling, Otitis media. (JPMA 76: 867; 2026)

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## Introduction

Middle ear infection, or otitis media (OM), is a common condition in humans that leads to hearing loss, and often necessitates medical consultation, antibiotic treatment and sometimes surgery.[1] In high-income countries, acute OM (AOM) is a leading reason for medical visits and treatments in children, often resulting in ear pain and fever. While severe complications, such as mastoiditis and meningitis, are uncommon, they are more frequent in low-income countries where AOM causes approximately 21,000 deaths annually. Worldwide, OM leads to hearing loss in 30 out of every 10,000 people, sometimes resulting in tympanic membrane perforation. OM with effusion (OME) causes conductive hearing loss, which can affect language development and academic performance, often occurring after AOM or viral infections.[2]

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Chronic suppurative OM (CSOM) is characterised by persistent inflammation of the middle ear and mastoid cavity, typically accompanied by continuous ear discharge through a perforated tympanic membrane or a ventilation tube. Chronic OME presents challenges in estimation due to its asymptomatic nature, with prevalence potentially reaching 20%, peaking around age 1. CSOM has a global incidence of 4.8 new episodes per 1,000 people annually, with 22% in children aged <5. While developed countries have seen a decline in OM incidence due to improved guidelines, developing countries and indigenous populations still face significant burdens, especially CSOM and its complications. Various host and environmental factors contribute to OM risk, including age, gender, genetics, environmental exposures and socioeconomic status (SES). In developing countries, factors like malnutrition and limited healthcare access exacerbate the risk. Early colonisation of the nasopharynx by bacterial pathogens, such as non-typeable haemophilus influenza (NTHi), streptococcus pneumoniae and moraxella (M.) catarrhalis significantly increases the likelihood of OM episodes.[3]

Significant advancements in OM have been made through pneumococcal conjugate vaccination and updated treatment guidelines for accurate diagnosis and prudent antibiotic use. These developments have reshaped OM's global epidemiology and clinical approach, offering future research directions.[4]

AOM rates ranged from 3.6 to 43.4 new episodes per 100 people annually, with sub-Saharan West Africa and Central Africa experiencing the highest rates. Children aged <5 accounted for 51% of new cases globally, with peak incidence between ages 1 and 4.[5] This risk varies depending on geographical location and pneumococcal conjugate vaccine (PCV) usage. Indigenous Australian infants aged 1-3 months are particularly vulnerable, facing distinct risks based on the pathogens they carry compared to non-Indigenous infants.[6]

Bacterial biofilms found in cases of CSOM and persistent OM pose significant challenges to treatment by resisting antibiotics and host immune responses. Immunisation targetting NTHi offers potential in combatting biofilm-related infections. Moreover, OM prevalent in paediatric populations can result in speech and behavioural issues due to the hearing loss induced by various pathogens, including *pseudomonas (P.) aeruginosa* and *staphylococcus (S.) aureus*, as well as other implicated pathogens, like *aspergillus (A.) species, proteus mirabilis* and *Klebsiella (K.) pneumonia*.[7] In countries like Pakistan, children typically have medical insurance or state-provided healthcare coverage. Indirect costs, such as lost work time for caregiving during a child's absence from school or daycare, vary.[8]

Although OM is a common cause of illness and hearing problems in children, there is very limited recent data from the Balochistan province of Pakistan on its prevalence, causative bacteria and antibiotic resistance pattern. The current study was planned to fill the gap in literature by assessing the antibiotic resistance profiles of bacterial pathogens isolated from patients with middle ear infections.

## Materials and Methods

The cross-sectional study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan, from November 2022 to December 2023, and comprised swab samples from middle ear infections collected from the Bolan Medical Complex Hospital, Quetta. After approval from the institutional ethics review committee, the sample size was calculated using the Cochran formula: $n=z^2p(1-p)/d^2$, where n represented the required sample size, Z was the 95% confidence level (1.96), p was the estimated proportion (0.5), and d was the margin of error (0.05).[9]

The samples were collected with pre-sterilised, pre-labelled cotton swabs, and transported to the laboratory under cold chain conditions. Data on patient gender, literacy, SES and age was recorded, encompassing all age groups, with a particular focus on vulnerable children experiencing AOM, CSOM, otitis externa (OE), or OME.

Participants of all age groups and both genders presenting with a clinical diagnosis of OM were eligible for enrolment. Only patients who voluntarily provided informed consent were included. To ensure microbiological validity, only specimens yielding confirmed bacterial growth from ear discharge or middle-ear aspirates were subjected to downstream analysis. Clinical diagnosis was established based on otoscopic findings, symptomatology and physician assessment. Patients who had received any systemic or topical antibiotic therapy within seven days prior to specimen collection were excluded to prevent false-negative cultures and ensure accurate pathogen recovery. Individuals diagnosed with fungal OM, or non-infectious aetiologies of ear discharge were also excluded. In addition, participants with incomplete clinical data, missing demographic information, or inadequate sample volume were excluded to maintain data integrity and analytical consistency.

The samples were initially streaked onto Brain Heart Infusion (BHI) medium and incubated at 37°C for 24 hours. After incubation, the bacterial cultures were streaked onto selective and differential media plates. Each isolate underwent triple cloning to ensure pure growth for gram staining and various biochemical tests, including sugar fermentation tests for glucose, maltose, mannitol, lactose and trehalose. Additionally, tests for indole, methyl red, voges-proskauer, simmons citrate, oxidase, catalase, motility and urease were conducted along with polymerase chain reaction (PCR) analysis. Pure colonies were used for organism confirmation through gram staining and various biochemical tests, including indole, simmons citrate, methyl red, voges-proskauer, oxidase, motility and catalase assays. Deoxyribonucleic acid (DNA) extraction was performed using a DNA purification kit, and the extracted DNA templates were stored at -20°C for future use. Specific primers were employed for each of the five strains to amplify their corresponding PCR fragments. The thermal cycling protocol included an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, primer-specific annealing at designated temperatures and times, an initial extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The PCR products were visualised using gel electrophoresis on a 1.5% agarose gel under ultraviolet (UV) light.

Antibiotic susceptibility testing was performed using the Kirby Bauer disc diffusion method on Mueller Hinton agar. Antibiotic discs (Oxoid Ltd., Thermo Fisher Scientific, United Kingdom) were used, and the manufacturer-provided lot numbers were recorded for all the discs (HiMedia). Antimicrobial susceptibility was evaluated in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[10] Mueller-Hinton agar plates (Oxoid, UK) were prepared by spreading a bacterial cell suspension (0.5 McFarland standard) across the surface, followed by incubation at 37°C for 24 hours. The sensitivity or resistance of the isolates to specific antimicrobial agents was assessed by measuring the inhibition zones.

Data was analysed using SPSS 20. Chi-square test was used as appropriate. P<0.05 was taken as significant.

## Results

Of 1,000 samples, 788 (78.8%) tested positive for pathogenic bacteria; 404 (40.4%) from male patients, 384 (38.4%) from female patients, and 474 (47.4%) from patients aged 1-15 years. *P. aeruginosa* was the most prevalent bacterium 192 (24.3%), followed by *S. aureus* 154 (19.5%), *escherichia (E.) coli* 99 (12.5%), *K. pneumoniae* 107 (13.5%) and *clostridium (C.) perfringens* 71 (9%). Age, gender and SES were significantly associated with pathogen positivity (Table 1).

The differentiation of the isolated bacteria was conducted based on colony morphology, gram staining, biochemical tests, and sugar fermentation tests (Table 2). Susceptibility test showed that the majority of bacterial isolates had high levels of resistance (Table 3).

PCR analysis confirmed the bacterial presence, identifying specific gene bands for each organism (Table 4).

**Table-1:** Comparison of basline and follow-up scores.

| Ear infection patients in Quetta, Balochistan. | | | |
|---|---|---|---|
| **Demographic parameters** | **Positive n (%)** | **Negative n (%)** | ***p*-value** |
| **Total Samples** | | | |
| 1,000 | 788 (78.8) | 212 (21.2) | |
| **Gender** | | | |
| Female | 404 (40.4) | 164 (16.4) | 0.001 |
| Male | 384 (38.4) | 47 (4.7) | |
| **Class** | | | |
| Higher | 20 (2) | 30 (3) | 0.002 |
| Middle | 224 (22.4) | 75 (7.5) | |
| Lower | 544 (54.4 ) | 107 (10.7) | |
| **Literacy rate** | | | |
| Literate | 220 (22) | 80 (8%) | 0.712 |
| Illiterate | 568 (56.8) | 132(13.2) | |
| **Age** (years) | | | |
| 1-15-years | 474 (47.4) | 125 (12.5) | 0.001 |
| 15-35 years | 202 (20.2) | 65 (6.5) | |
| 35-50 years | 112 (11.2) | 25 (2.5) | |

**Table-2:** Biochemical profiling of the bacterial pathogens recovered from middle ear-infected samples.

| | **Biochemical tests of isolated bacteria** | | | | |
|---|---|---|---|---|---|
| **Tests** | ***Staphylococcus aureus*** | ***Pseudomonas aeruginosa*** | ***Escherich coli*** | ***Klebsiella pneumoniae*** | ***Clostridium perfringens*** |
| Indole test | - | - | + | - | - |
| Methyl red test | + | - | + | - | + |
| Voges-Proskauer test | + | - | - | + | - |
| Simmons citrate test | - | + | - | + | + |
| Motility test | - | + | + | - | - |
| Catalase test | + | + | + | + | - |
| Oxidase test | - | + | - | - | - |
| Urease test | - | - | - | + | + |
| | **Sugar fermentation tests** | | | | |
| Glucose test | + | + | + | + | + |
| Maltose test | + | - | + | + | + |
| Mannitol test | + | + | + | + | - |
| Trehalose test | - | - | + | + | + |
| Lactose test | + | - | + | + | + |

**Table-3:** Antibiotic susceptibility testing of the isolates analysed.

| **Antibiotics** | **Conc** (µg) | ***S. aureus*** n=10 [n (%)] | | ***P. aeruginosa*** n=10 [n (%)] | | ***E. coli*** n=10 [n (%)] | | ***K. pneumonia*** n=10 [n (%)] | | ***C. perfringens*** n=10 [n (%)] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** |
| Erythromycin | 25 | 3(30) | 7(70) | 9(90) | 1(10) | 9(90) | 1(10) | 2(20) | 8(80) | 2(20) | 8(80) |
| Meropenem | 25 | 2(20) | 8(80) | 9(90) | 1(10) | 3(30) | 7(70) | 2(20) | 8(80) | 3(30) | 7(70) |
| Trimethoprim | 10 | 7(70) | 3(30) | 8(80) | 2(20) | 2(20) | 8(80) | 3(30) | 7(70) | 1(10) | 9(90) |
| Colistin Sulfate | 30 | 8(80) | 2(20) | 3(30) | 7(70) | 4(40) | 6(60) | 3(30) | 7(70) | 4(40) | 6(60) |
| Vancomycin | 5 | 2(20) | 8(80) | 8(80) | 2(20) | 8(80) | 2(20) | 8(80) | 2(20) | 1(10) | 9(90) |
| Metronidazole | 30 | 9(90) | 1(10) | 9(90) | 1(10) | 8(80) | 2(20) | 8(80) | 2(20) | 2(20) | 8(80) |
| Tetracycline | 10 | 3 (30) | 7(70) | 9(90) | 1(10) | 7(70) | 3(30) | 7(70) | 3(30) | 3(30) | 7(70) |
| Lincomycin | 5 | 1(10) | 9(90) | 8(80) | 2(20) | 8(80) | 2(20) | 9(90) | 1(10) | 1(10) | 9(90) |
| Spectinomycin | 10 | 2(20) | 8(80) | 8(80) | 2(20) | 1(10) | 9(90) | 3(30) | 7(70) | 2(20) | 8(80) |
| Pencilline G | 30 | 8(80) | 2(20) | 9(90) | 1(10) | 8(80) | 2(20) | 9(90) | 1(10) | 9(90) | 1(10) |

*S: Staphylococcus, P: Pseudomonas, E: Escherichia, K: Klebsiella, C: Clostridium.*

**Table-4:** Primers used for bacteria isolation.

| Organisms | Direction | Primer sequence | Bp size | Annealing temperature |
|---|---|---|---|---|
| *Escherichia coli*[23] | F | 5`-GGGAGTAAAGTTAATACCTTTGC-3` | 204bp | 45 sec at 59.8°C |
| | R | 5`-CTCAAGCTTGCCAGTATCAG-3` | | |
| *Staphylococcus aureus*[24] | F | 5`ACGGTCTTGCTGTCACTTATA-3` | 257bp | 45 sec at 57°C |
| | R | 5`TACACATATGTTCTTCCCTAATAA-3` | | |
| *Pseudomonas aeruginosa*[25] | F | 5`-ATGGAAATGCTGAAATTCGGC-3` | 504bp | 60 sec at 55°C |
| | R | 5`-CTTCTTCAGCTCGACGGGACG-3` | | |
| *Klebsiella Pneumoniae*[26] | F | 5`-TCTGAGAGGATGACCAGCCA-3` | 525bp | 54 sec at 60°C |
| | R | 5`-GTTTACGGCGTGGACTACCA-3` | | |
| *Clostridium perfringens*[27] | F | 5′-GGAGATGGTTGGATATTAGG-3` | 233bp | 30 sec at 55°C |
| | R | 5′-GGACCAGCAGTTGTAGATA-3` | | |

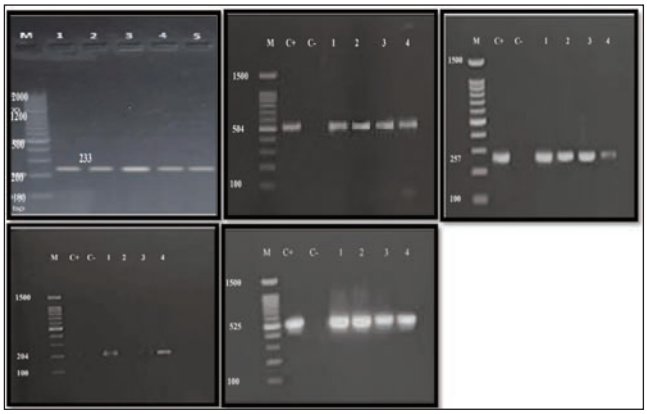

**Figure:** a) Polymerase chain reaction (PCR)-based identification of *staphylococcus (S.) aureus* produced a specific size of the 257-bp fragment of the 16S ribosomal ribonucleic acid (rRNA) gene, (b) *Pseudomonas (P.)aeruginosa* produced a specific size of the 504-bp fragment of the outer membrane peptidoglycan-associated lipoprotein (OprL) gene, (c) *Escherichia (E.) coli* produced a specific size of the 204-bp fragment of the 16S rRNA gene, (d) *Klebsiella (K.)* pneumoniae produced a specific size of the 525-bp fragment of the 16S rRNA gene, and (e) *Clostridium (C.) perfringens* produced a specific size of 233-bp fragment of the enterotoxin gene.

Polymerase chain reaction analysis confirmed the bacterial presence, identifying specific gene bands for each organism as shown in (Figure 1).

## Discussion

The current study focused on assessing the incidence and antibiotic resistance patterns of bacterial isolates from middle ear infections in Quetta by examining 1,000 samples. The positive rate was 78.8% for pathogenic bacteria. *P. aeruginosa* was the most commonly isolated pathogen, followed by *S. aureus*, *K. pneumoniae, E. coli* and *C. perfringens*. These findings corroborate earlier research highlighting *S. aureus* as a prevalent pathogen in ear infections. The current also showed the age distribution of AOM, which is frequently observed in children aged 6-24 months. This age group is especially vulnerable to OM, with about 80% experiencing it at some point, and a significant incidence of OME occurring before school age.[11]

Regarding gender, the current study noted a higher prevalence of ear infections among female patients (40.4%) compared to male patients (38.4%). This observation was consistent with a 2018 study.[12]

The current study also explored the SES factor influencing ear infections, showing a higher prevalence in lower socioeconomic classes (54.4%). This finding aligns with Guys et al.[13] The age distribution of middle ear infections in the current study showed the highest infection rate among individuals aged 1-15 years (50.2%), followed by those aged 15-35 years (18.4%) and 35-55 years (10.2%). This pattern was in line with earlier observations attributing higher prevalence in younger age groups to factors such as underdeveloped immune defences and anatomical predispositions.[14]

The morphological characteristics and biochemical test results for the pathogens in the current study were consistent with previous studies.[15-18]

In terms of antimicrobial resistance, the isolated organisms exhibited diverse resistance profiles in the current study. *S. aureus* demonstrated high sensitivity to vancomycin, meropenem, erythromycin, spectinomycin, lincomycin, and oxytetracycline, while showing marked resistance to clindamycin, trimethoprim, metronidazole, and penicillin G. These findings were in agreement with the resistance trends reported in 2020.[19]

P. aeruginosa and K. pneumoniae demonstrated high resistance against multiple antibiotics, as reported earlier.[20] In contrast, *E. coli* showed sensitivity to meropenum, spectinomycin, colistin sulfate, and trimethoprim, which was also reported by Badr et al. in 2022.[21] *C. perfringens* displayed sensitivity to erythromycin, but resistance to penicillin G and metronidazole in the current study, which was consistent with Akhi et al.[22]

Genomic DNA extraction played a crucial role in the diagnostic process, enabling the identification of specific genes associated with each pathogen. These results aligned with previous studies regarding gene fragment sizes for *E.coli P. aeruginosa, S. aureus, K. pneumoniae* and *C. perfringens* (Table 4).[23-27]

Antibiotic stewardship in OM focusses on using antibiotics only when truly needed to prevent resistance. Preventive measures, such as pneumococcal and influenza vaccination, good hygiene, avoiding smoke exposure, and breastfeeding, help reduce the risk of ear infections and decrease unnecessary antibiotic use.

The current study has limitations as it was conducted at a

single tertiary care hospital, which may not represent the broader population, especially in rural or under-resourced areas. Furthermore, due to limited resources, molecular methods for bacterial identification and resistance gene detection were not employed, restricting the ability to detect resistant strains that may not express phenotypic resistance.

## Conclusion

Middle ear infections were most prevalent in patients aged 1-15 years. The bacterial isolates demonstrated multidrug resistance, highlighting the importance of enhancing efforts to isolate microorganisms and determine their susceptibility patterns in order to improve the treatment outcomes for otitis media.

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**Author Contribution:**
**LB:** Literature search, writing and performed the experiments.
**MKT:** Supervision and design.
**SA:** Concept and design.
**SK:** Analysed the data.