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Original article

Identification and Antibiotic Susceptibility Patterns of Bacterial Isolates from External Ear Infections in Children Aged 0–3 Years at Al-Shahid Emhamed Al-Magarif Educational Hospital, Ajdabiya

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Abstract
Ear infections represent one of the most prevalent otolaryngological problems in pediatric populations, with potential complications such as hearing loss, delayed speech development, and recurrent morbidity. This study aimed to isolate and identify bacterial species associated with external ear infections in children aged 0–3 years and to assess their antibiotic susceptibility patterns. A total of 80 ear swab specimens were collected from pediatric patients admitted to Al-Shahid Emhamed Al-Magarif Educational Hospital, Ajdabiya, Libya, between July 2023 and January 2024. Standard culture methods, Gram staining, and biochemical assays were employed for bacterial identification, while antibiotic susceptibility was determined using the disk diffusion method. Five bacterial species were isolated: *Staphylococcus aureus* (35%), *Pseudomonas aeruginosa* (27.5%), *Streptococcus pneumoniae* (17.5%), *Staphylococcus epidermidis* (10%), and *Escherichia coli* (10%). *Staphylococcus aureus* exhibited high susceptibility to imipenem (80%), norfloxacin (78%), amikacin, and levofloxacin (75%), with resistance to chloramphenicol. *Pseudomonas aeruginosa* showed high susceptibility to gentamicin (82%), tobramycin, and chloramphenicol (75%), but was resistant to multiple β-lactam antibiotics. *Streptococcus pneumoniae* demonstrated high susceptibility to amikacin (96%) but resistance to norfloxacin. *Staphylococcus epidermidis* exhibited high susceptibility to imipenem (96%), levofloxacin and trimethoprim-sulfamethoxazole (87%), and amikacin, tobramycin, cefuroxime, and meropenem (75%), with resistance to chloramphenicol. *Escherichia coli* was sensitive only to amikacin (80%) while resistant to other tested antibiotics. These findings confirm the predominance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in pediatric external ear infections and highlight the growing challenge of multidrug resistance. Culture-guided therapy, rational antibiotic prescribing, and regular surveillance are essential to improve treatment outcomes and limit antimicrobial resistance.
Keywords: Otitis Externa, Pediatric Infections, Bacterial Isolates, Antibiotic Susceptibility, Ajdabiya.

Introduction

Ear infections are among the most common disorders affecting both children and adults, with their prevalence varying across countries and populations. They are broadly classified into otitis media (middle ear infections) and otitis externa (external ear infections), both of which are predominantly caused by bacterial pathogens. The duration of these infections may range from two weeks to over three months, manifesting as either acute or chronic conditions. Globally, ear infections represent a major health concern, with approximately 60% of affected individuals experiencing some degree of hearing loss. Such complications can contribute to speech impairments, delayed language development, and social interaction difficulties. Children, particularly infants, are more susceptible than adults due to the anatomical characteristics of their Eustachian tubes, which are shorter and thus facilitate the entry of microorganisms from the nasopharynx [1].

Several bacterial species have been implicated in ear infections. *Pseudomonas* and *Klebsiella* are among the most pathogenic, while *Staphylococcus* and *Streptococcus* spp. are also frequently isolated [2,3]. The ear's structural exposure through the auricle and the auditory canal, which connects directly to the pharynx, further predisposes it to microbial colonization. *Staphylococcus aureus*, despite being a commensal organism commonly found on human skin and mucosa, is recognized as a primary pathogen in middle ear infections, accounting for nearly half of pediatric cases in certain regions such as Al-Diwaniyah, Iraq [4]. The clinical presentation of ear infections varies according to the causative agent, which directly influences therapeutic decisions.

This variability, however, has contributed to inappropriate antibiotic use and the alarming rise of antimicrobial resistance [5], now regarded by the World Health Organization as a global health threat [6].

Previous studies have suggested that gram-positive bacteria associated with ear infections typically originate from the nasopharyngeal cavity, whereas gram-negative enteric bacteria often result from contamination of the auditory canal [7]. Furthermore, many children with ear infections have a prior history of respiratory tract infections, supporting the view that pathogenic bacteria are commonly derived from the respiratory flora [8]. The present study aims to isolate and identify bacterial species from the external ear of children aged 0–3 years and to evaluate their antibiotic susceptibility profiles at Al-Shahid Emhamed Al-Magarif Educational Hospital, Ajdabiya.

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Methods

Specimen Collection

Eighty (80) ear swabs were collected from pediatric and neonatal patients at Al-Shahid Mohamed Al-Magrief Teaching Hospital between July 15, 2023, and January 4, 2024. Swabs were obtained aseptically from the external auditory canal and transported immediately to the microbiology laboratory for bacterial culture and analysis.

Bacterial Isolation and Diagnosis

Swabs were inoculated onto nutrient agar, blood agar, MacConkey agar, and chocolate agar (Liofilchem, Italy). The inoculated plates were incubated aerobically at 37 °C for 18–24 hours before evaluation.

Biochemical tests

Following bacterial isolation, standard biochemical tests were performed to identify and differentiate bacterial isolates [9].

Indole test: Tryptone water medium (Liofilchem, Italy) was inoculated with bacterial isolates and incubated at 37 °C for 24 hours. After incubation, 0.5 ml of Kovac's reagent was added. The development of a red ring indicated a positive result, whereas the absence of color change indicated a negative result.

Citrate test: Simmons citrate agar slants (Liofilchem, Italy) were inoculated and incubated at 37 °C for 24 hours. A color change of the medium from green to blue indicated a positive result, while no change (medium remaining green) indicated a negative result.

Coagulase test: This test was performed to differentiate *Staph. aureus* from another *Staphylococcus spp*. A drop of sterile saline was placed on a clean glass slide, inoculated with the test organism, and mixed with a drop of plasma. The presence of visible clumping within a few seconds indicated a positive result, while the absence of clumping indicated a negative result.

Catalase test: A small portion of a bacterial colony was placed on a clean glass slide and mixed with one drop of 3% hydrogen peroxide. Immediate bubble formation indicated a positive result, whereas the absence of bubbles indicated a negative result. This test differentiates catalase-positive staphylococci from catalase-negative streptococci.

Oxidase test: Filter paper was impregnated with Tetramethyl-p-phenylenediamine dihydrochloride reagent. A colony was applied to the reagent-soaked paper, and the reaction was observed within 10 seconds. A purple color indicated a positive result, while no color change was recorded as negative.

Mannitol fermentation test: Mannitol Salt Agar (Liofilchem, Italy) was inoculated with bacterial isolates and incubated at 37 °C for 24 hours. A positive result was indicated by a color change of the medium from pink to yellow, showing mannitol fermentation. No color change indicated a negative result. This test was particularly useful in differentiating *Staph. aureus* (mannitol-fermenting) from *Staph. epidermidis* and *Staph. saprophyticus*, which do not ferment mannitol.

Antibiotic susceptibility testing

Antibiotic susceptibility of Gram-positive and Gram-negative bacterial isolates was determined using the disk diffusion method [10] on Mueller-Hinton Agar. The medium was inoculated with the standardized bacterial suspension adjusted to a 0.5 McFarland standard. Twelve antibiotics were tested (Table 1) following standard procedures. Plates were incubated at 37 °C for 24 hours. After incubation, inhibition zone diameters were measured, and the drug susceptibility patterns of the isolates were examined and interpreted according to CLSI guidelines (2023).

Table 1. Antibiotics used for susceptibility testing

No.	Antibiotic	Abbreviation	Manufacturer	Concentration (µg/disc)
1	Amikacin	AK30	Liofilchem	30
2	Gentamicin	GN30	Liofilchem	30
3	Tobramycin	TOB30	Liofilchem	30
4	Levofloxacin	LEV5	Liofilchem	5
5	Norfloxacin	NOR10	Liofilchem	10
6	Amoxicillin-clavulanic acid	AMC30	Liofilchem	30
7	Ceftizoxime	ZOX30	Liofilchem	30
8	Cefoperazone	CPZ75	Liofilchem	75
9	Imipenem	IPM10	Liofilchem	10
10	Meropenem	MEM10	Liofilchem	10
11	Trimethoprim/Sulfamethoxazole	SXT25	Liofilchem	25
12	Chloramphenicol	CHL30	Liofilchem	30

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Results

Age and gender distribution

A total of 80 ear swab specimens were collected from pediatric and neonatal patients admitted to Al-Shahid Al-Maqreef Teaching Hospital. The age distribution revealed that 47.5% of participants were between 1 month and less than 1 year, 31.3% were between 1 day and less than 1 month, and 21.3% were over one year old, as illustrated in Table 2. Regarding gender, 55% of participants were male and 45% were female, as illustrated in Table 3.

Table 2. Age distribution of study participants

Age group	Frequency (n)	Percentage (%)
Day to less than 31 days 1	25	31.3%
1 month to less than 1 year	38	47.5%
≥1 year	17	21.3%

Table 3. Gender distribution of study participants

Gender	Frequency (n)	Percentage (%)
Male	44	55%
Female	36	45%

Bacterial isolates

The results of bacterial cultures showed that *Staph. aureus* was the most prevalent isolate (35%), followed by *P. aeruginosa* (27.5%), *S. pneumoniae* (17.5%), *Staph. epidermidis* (10%), and *E. coli* (10%), as illustrated in (Table 4).

Table 4. Distribution of bacterial isolates by frequency and percentage

Bacterial Isolates	Frequency (n)	Percentage (%)
Staph. aureus	28	35%
P. earuginosa	22	27.5%
S. pneumoniae	14	17.5%
Staph. epidermidis	8	10%
E. coli	8	10%
Total	80	100%

Biochemical identification

Bacterial isolates were identified based on standard biochemical tests [9], as shown in (Table 5), (Figure 1).

Table 5: Biochemical tests and identification of bacterial isolates

Bacterial isolates	Biochemical tests and Identification						
	Gram stain	Indole	Citrate	Coagulase	Catalase	Oxidase	Mannitol Fermentation
Staph. aureus	+	-	+	+	+	-	+
P. aeruginosa	-	-	+	-	+	+	+
S. pneumoniae	+	-	-	-	-	-	-
Staph. epidermidis	+	-	-	-	+	-	-
E. coli	-	+	-	-	+	-	+

Note: + = positive reaction; – = negative reaction

Antibiotic Susceptibility

The antibiotic susceptibility profile of bacterial isolates is presented in (Table 6), (Figure 5). *Staph. aureus* exhibited high susceptibility to imipenem (IPM, 80%), norfloxacin (NOR, 78%), amikacin, and levofloxacin (AK& LEV, 75%). Moderate susceptibility was observed to gentamicin and tobramycin (GN& TOB, 60%), and low susceptibility to amoxicillin–clavulanate (AMC, 46%), cefoperazone and trimethoprim-sulfamethoxazole (CPZ& SXT, 42%), ceftizoxime (ZOX, 39%), and meropenem (MEM, 28%). Whereas there was resistance to chloramphenicol (CHL). *P. aeruginosa* demonstrated high susceptibility to gentamicin (GN, 82%), tobramycin and chloramphenicol (TOB& CHL, 75%), and low susceptibility to amikacin (AK, 46%), norfloxacin (NOR, 14%), and levofloxacin (LEV, 10%), but resistant to amoxicillin-clavulanate (AMC),

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cefuroxime (ZOX), cefoperazone (CPZ), imipenem (IPM), meropenem (MEM), and trimethoprim-sulfamethoxazole (SXT).

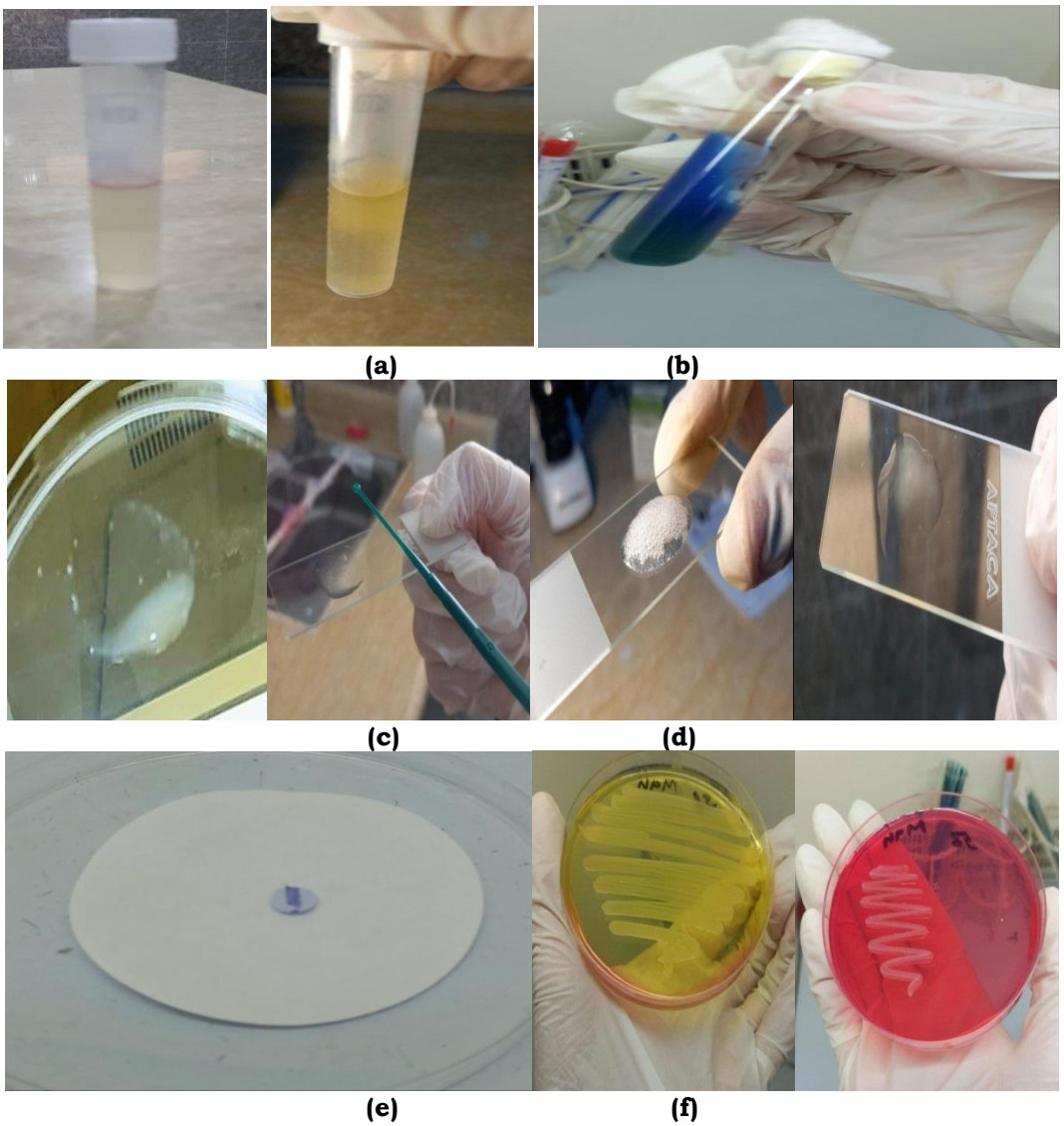

Figure 1. Biochemical identification of bacterial isolates: (a) Indole test — P^+ (red ring)/N^- (no color); (b) Citrate test — P^+ (blue)/ N^- (green); (c) Coagulase test — P^+ (clumping)/N^- (no clumping); (d) Catalase test — P^+ (bubbling)/N^- (no bubbles); (e) Oxidase test — P^+ (purple)/ N^- (no color change); (f) Mannitol Fermentation — P^+(yellow)/N^-(pink).

S. pneumoniae showed high susceptibility to amikacin (AK, 96%), moderate susceptibility to amoxicillin-clavulanate, cefuroxime, imipenem, meropenem, and trimethoprim-sulfamethoxazole (AMC, ZOX, IPM, MEM& ZXT, 64%), and tobramycin (TOB, 57%), and low susceptibility to gentamicin (GN, 42%), cefoperazone and chloramphenicol (CPZ& CHL, 35%), and levofloxacin (LEV, 28%). Whereas there was resistance to norfloxacin (NOR). *Staph. epidermidis* exhibited high susceptibility to imipenem (IPM, 96%), levofloxacin and trimethoprim-sulfamethoxazole (LEV& SXT 87%), amikacin, tobramycin, cefuroxime and meropenem (AK, TOB, ZOX& MEM, 75%), moderate susceptibility to norfloxacin (NOR, 62%), and amoxicillin-clavulanate (AMC, 50%), and low susceptibility to gentamicin (GN, 37%), and cefoperazone (CPZ, 25%) with strong resistance to chloramphenicol (CHL). *E. coli* showed high susceptibility to amikacin (AK, 80%), while remaining resistant to the other antibiotics tested.

Table 6. Antibiotic susceptibility profiles of bacterial isolates

Antibiotics	Staph. aureus	P. aeruginosa	S. pneumoniae	Staph. epidermidis	E. coli
AK 30µg	75%	46%	96%	50%	80%
GN 30µg	60%	82%	42%	37%	R
TOB 30µg	60%	76%	57%	75%	R
LEV 5µg	75%	10%	28%	87%	R
NOR 10µg	78%	14%	R	62%	R
AMC 30µg	46%	R	64%	50%	R

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ZOX 30µg	39%	R	64%	75%	R
CPZ 75µg	42%	R	35%	25%	R
IPM 10µg	80%	R	64%	96%	R
MEM 10µg	28%	R	64%	75%	R
SXT 25µg	39%	R	64%	87%	R
CHL 30µg	R	75%	35%	R	R

Discussion

The present study investigated bacterial isolates from the external auditory canal of pediatric and neonatal patients, alongside their antibiotic susceptibility profiles. A total of 80 ear swab specimens were collected and analyzed using standard microbiological and biochemical techniques, including Gram staining, catalase, coagulase, oxidase, indole, citrate utilization, and mannitol fermentation [9]. These methods allowed reliable differentiation between Gram-positive and Gram-negative bacteria and facilitated accurate species identification, consistent with established protocols. *Staph. aureus* was the most frequently isolated bacterium, accounting for 35% of cases. This finding aligns with its widespread presence as a natural colonizer of human skin and the oropharyngeal cavity, from which it can migrate to the auditory canal via the relatively short Eustachian tube in children [11].

P. aeruginosa was the second most prevalent isolate (27.5%), reflecting its recognized role as a common nosocomial pathogen in pediatric otologic infections, supported by its ability to persist in moist environments and form biofilms, which enhance pathogenicity and resistance [12]. *S. pneumoniae* represented 17.5% of isolates, originating from the oropharynx and serving as a frequent causative agent of pediatric otitis, consistent with previous reports [13]. Other bacterial isolates include *Staph. epidermidis* (10%) and *E. coli* (10%). *Staph. epidermidis*, a known skin commensal including the tympanic membrane epithelium, is typically non-pathogenic but can occasionally act opportunistically [14]. The presence of *E. coli*, a Gram-negative enteric bacterium, likely reflects fecal contamination resulting from inadequate hygiene or improper aural care, which emphasizes the importance of preventive measures in pediatric populations [15]. Overall, these results corroborate findings indicating that *Staph. aureus* and *P. aeruginosa* are the predominant pathogens in pediatric external ear infections, followed by *S. pneumoniae* and other commensal organisms [14, 16].

Antibiotic susceptibility testing revealed distinct susceptibility and resistance patterns among the isolates. *Staph. aureus* exhibited susceptibility to IPM, NOR, AK, LEV, GN, TOB, AMC, CPZ, SXT, ZOX, and MEM. Resistance to chloramphenicol (CHL) was observed, consistent with previous reports [17]. *P. aeruginosa* demonstrated susceptibility to GN, TOB, CHL, AK, NOR, and LEV, but showed resistance to multiple β-lactam antibiotics, including AMC, ZOX, CPZ, IPM, MEM, and SXT, highlighting its multidrug-resistant potential [17,18]. *S. pneumoniae* showed susceptibility to amikacin AK, AMC, ZOX, IPM, MEM, ZXT, TOB, GN, CPZ, CHL, and LEV. Whereas there was resistance to NOR [7, 19]. *Staph. epidermidis* exhibited susceptibility to IPM, LEV, SXT, AK, TOB, ZOX, MEM, NOR, AMC, GN, and CPZ, while being resistant to chloramphenicol CHL [20]. *E. coli* showed susceptibility to AK, while remaining resistant to the other antibiotics tested [21].

The multidrug resistance patterns observed in the present study can be contextualized by comparing with regional and global trends. A study conducted in Ethiopia reported that 72.45% of pediatric ear bacterial isolates were resistant to at least one antibiotic, with 80.09% exhibiting multidrug resistance [22]. Similarly, surveillance data from the Eastern Mediterranean region published by the World Health Organization indicate a rising prevalence of antimicrobial resistance among common pediatric pathogens [23]. Furthermore, a systematic analysis identified *Staph. aureus* and *P. aeruginosa* as major contributors to the regional burden of antimicrobial resistance, reinforcing the importance of continuous surveillance and targeted antibiotic stewardship programs. This highlights the need for ongoing monitoring and evidence-based antimicrobial policies for pediatric pathogens [24].

Conclusion

This study demonstrates that *Staph. aureus* and *P. aeruginosa* are the predominant bacterial pathogens in pediatric external ear infections, followed by *S. pneumoniae* and commensal organisms such as *Staph. epidermidis* and *E. coli*. The observed antibiotic resistance patterns, particularly among *P. aeruginosa*, underscore the importance of culture-guided therapy and rational antibiotic use in pediatric populations. Moreover, the detection of enteric organisms highlights the critical role of proper hygiene and oral care in reducing infection risk. Overall, these findings emphasize the necessity for continuous monitoring of bacterial isolates and their susceptibility profiles to guide effective treatment strategies and limit the emergence of multidrug-resistant pathogens.

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Conflicts of Interest

The authors declare no conflicts of interest

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