

RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF BACTERIA CAUSING EAR INFECTIONS AND ASSOCIATED RISK FACTORS AMONG PATIENTS ATTENDING ENT CLINICS IN ADEN, YEMEN

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Abstract

Ear infections represent a critical, neglected public health crisis in developing countries, severely impacting millions of children and adults. This study aimed to identify the bacterial etiological agents of ear infections among patients attending ENT clinics in Aden City and to determine the risk factors associated with bacterial pathogens. A total of 181 ear swab samples were collected from August to October 2025 and cultured on appropriate media. The bacterial isolates were identified using colony characteristics, Gram staining, and standard biochemical tests. Out of the 181 samples, 171 (94.5%) were culture-positive. *Pseudomonas aeruginosa* was the most frequently isolated organism (45.6%), followed by *Staphylococcus aureus* (23.9%), *Escherichia coli* (15.2%), *Klebsiella spp.* (7.6%), *Proteus spp.* (4.7%), and *Streptococcus spp.* (1.8%), whereas *Enterobacter spp.* and *Citrobacter spp.* were the least common (0.6% each). The highest positive infection rate was (42.1%) in children ≤ 10 years old. Most positive infections occurred in females (60.2%) compared to males (39.8%). Positive infections were more common among the illiterate group (43.3%) and among urban residents (56.1%). Exposure to water appeared as the most significant risk factor (98.1%). The *P. aeruginosa* was the most common pathogen in ear infections. Early diagnosis, appropriate treatment, and strengthened health education and prevention are recommended, especially for children and other high-risk groups.

Keywords: Ear infection; Bacterial isolates; Risk factors; Aden governorate.

1. Introduction

Ear infection is a common disorder affecting both children and adults, with prevalence varying among countries due to factors such as pathogenic microorganisms, frequent antibiotic use, health care services, and age [1, 2]. Otitis media is an inflammatory disease of the middle ear that may cause temporary or permanent hearing impairment due to fluid effusion or pathological changes in the tympanic membrane [3]. Otitis externa involves infection of the auricle and external auditory canal, possibly involving the tympanic membrane [4]. Ear infections may be acute or chronic [5]. Although viral and fungal agents may be involved, bacterial pathogens are the primary causes of ear infections. The most common bacterial isolates include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli* [6,7]. Several factors have been

associated with an increased risk of ear infections, including inappropriate ear manipulation, exposure to water during swimming, low educational and socioeconomic status, and overcrowded living conditions [8, 9]. Accumulating clinical and microbiological data demonstrate a widespread distribution of these infections across multiple Yemeni governorates, including Sana'a, Hadhramout, and Al-Hodeidah, frequently manifesting as acute otitis media [10-12]. Epidemiological evaluations indicate that these infections affect various demographic groups, with a pronounced burden observed among young adults and males [13]. Furthermore, laboratory diagnostics reveal that the vast majority of documented cases are primarily driven by active bacterial etiologies [11,13]. Consensus across multi-regional studies identifies *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the most predominant and frequently isolated pathogens from infected ear discharges nationwide [10-14]. Other Gram-

negative bacilli, such as *Proteus mirabilis* and *Klebsiella species*, are also encountered as contributing pathogens, albeit at varying isolation rates [10,11]. In Aden, and specifically in the study area, there is not enough information to clarify the prevalence of ear infections, so the aim of this study was to identify the bacterial isolates that infect the ear, in addition to understanding the risk factors associated with ear infections.

2. Materials and Methods

2.1. Study design:

A cross-sectional descriptive and analytical study was carried out over a 3-month period from August to October 2025. The study included patients from several ENT clinics at Al Jumhuriya Hospital, which is located in the Khormaksar District. The study also included a group of private clinics located in Al-Mansoura District, such as the ENT clinics at Al-Burj Medical Center, Dr. Saleh Al-Shuaibi Clinic, Dr. Salah Bin Atif Clinic, and Al-Nahdha Medical Center.

2.2. Study Population:

Ear swab specimens were collected from all eligible patients clinically diagnosed with ear infection who attended the ENT clinics during the study period. Ear discharges were aseptically collected by the attending physicians. A total of 181 patients were included in the study.

2.3. Data collection:

Data were collected using a structured questionnaire developed by the authors based on questionnaires used in previously published studies [15,16], with modifications made to suit the objectives and context of the present study. The questionnaire was used to collect relevant demographic, clinical, and behavioral data, including age, sex, residence, and education level. It also assessed medical history and risk factors such as chronic diseases, prior ear infections or surgeries, swimming and ear hygiene practices, antibiotic self-medication, use of hearing-related devices, recent water exposure or ear treatments, and immunocompromising conditions.

2.4. Sample Collection, Culture, and Identification:

Ear swab samples were aseptically collected from infected patients after cleaning the external ear with sterile saline using sterile cotton swabs inserted into the ear canal without contamination. Relevant clinical data, including prior antibiotic or ear drop use, were recorded. Swabs were immediately placed in sterile transport media and delivered to the microbiology laboratory within one hour [17,18]. Bacterial isolation was performed on MacConkey, Blood, and Chocolate agar prepared and sterilized according to standard protocols. Plates were inoculated using sterile techniques and incubated at 37°C for 24 hours. Colony morphology and

pigmentation were recorded for preliminary identification [18-20]. Further identification was carried out using Gram staining and standard biochemical tests, including oxidase, catalase, coagulase, citrate utilization, Kligler Iron Agar (KIA), urease, and SIM tests, following established microbiological methods [19, 21-23].

2.4.1. Inclusion Criteria:

This includes individuals with a clinically confirmed ear infection presenting at ENT clinics, regardless of age or sex, who provide informed consent and from whom an ear swab or discharge sample can be successfully obtained.

2.4.2. Exclusion Criteria:

Conversely, individuals presenting without clinical evidence of infection, having received prior antibiotic therapy before sample collection, unable to yield an adequate specimen, or declining participation are excluded.

2.5. Statistical Analysis:

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). The Chi-square test was used to examine associations between categorical variables, including the distribution of infections across age groups, the most frequently isolated bacteria, distribution by gender, education levels, and place of residence, as well as the relationship between risk factors and bacterial infection. Results were considered statistically significant at $P \leq 0.05$.

3. Results

3.1. Analysis of Patients and Data:

A total of 181 clinical samples from patients with suspected ear infections were evaluated. Regarding age distribution, the highest proportion of cases was observed in the ≤ 10 years age group 75 (41.43%), followed by the 21–30 years group 33 (18.23%), the ≥ 51 years group 25 (13.8%), the 11–20 years group 22 (12.15%), and the 31–40 years group 16 (8.83%). Conversely, the 41–50 years cohort exhibited the lowest frequency 10 (5.52%). This variation across age groups was highly statistically significant ($p < 0.001$) (Table 1). In terms of gender, females constituted the majority of the study population 107 (59.1%), compared to males 74 (40.9%), a difference that was statistically significant ($p = 0.014$) (Table 1). Geographically, a higher number of samples originated from patients residing in urban areas 101 (56%), than in rural regions 80 (44%); however, this distribution did not reach statistical significance ($p = 0.119$). Analysis of educational background revealed that illiterate participants represented the largest group 78 (43.1%), followed by those with secondary or higher education 58 (32%) and primary education 45 (24.9%),

showing a statistically significant association ($p = 0.010$) (Table 1).

Table 1: Distribution of ear infection patients according to demographic characteristics (age groups, gender, residence and education level).

Studied variables		No. of Patients		P- value
		No.	%	
Age groups (years)	≤10	75	41.43	0.001
	11-20	22	12.15	
	21-30	33	18.23	
	31-40	16	8.83	
	41-50	10	5.52	
	≥51	25	13.8	
	Total	181	100	
Gender	Male	74	40.9	0.014
	Female	107	59.1	
	Total	181	100	
Residence	Urban	101	56	0.119
	Rural	80	44	
	Total	181	100	
Education level	Illiterate	78	43.1	0.010
	Primary	45	24.9	
	Secondary or higher	58	32	
	Total	181	100	

3.2. Frequency of bacterial isolates:

Figure 1 shows that a total of 171 samples (94.5%) showed bacterial growth. *Pseudomonas aeruginosa* was the most common isolate 78(45.6%), followed by *Staphylococcus aureus* 41(23.9%), *Escherichia coli* 26 (15.2%), *Klebsiella* spp. 13 (7.6%), *Proteus* spp. 8(4.7%) and *Staphylococcus* spp. 3(1.8%), whereas *Enterobacter* spp. and *Citrobacter* spp. were lowest bacteria with 1 (0.6%). The distribution of bacterial species among patients was highly uneven and statistically significant ($p < 0.001$)

3.3. Distribution of bacterial isolates in different age groups and gender:

Analysis of the age distribution among positive cases revealed that the highest incidence of infection was recorded in the youngest age group (≤10 years) 72 (42.1%), followed by the 21–30 years cohort 29 (16.9%). The groups aged 11–20 years and ≥51 years exhibited comparable infection counts of 22 (12.9%), while 16(9.4%) cases were reported in the 31–40 years range. Conversely, the lowest frequency of positive infections was observed in the 41–50 years category 10(5.8%). These variations in infection rates across different age brackets were highly statistically significant ($p < 0.001$) (Table 2). The results also revealed that the most positive infections occurred in females 103 (60.2%), compared with males 68 (39.8%). This was highly statistically significant ($P = 0.007$). (Table 2).

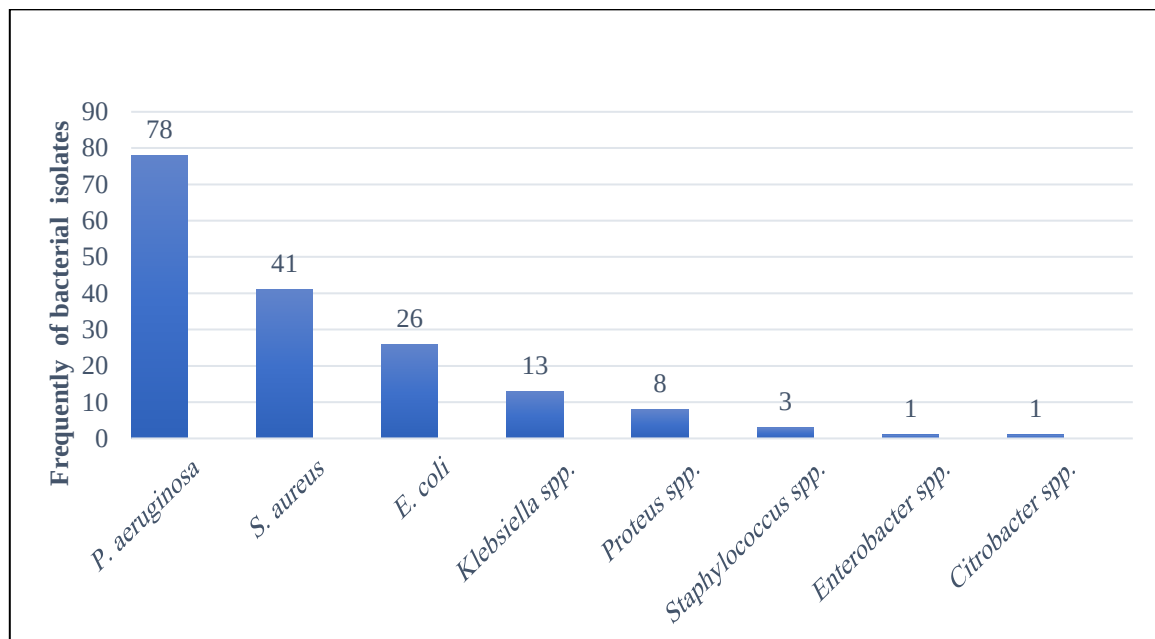


Fig. 1: Frequency of bacterial isolates from patients with ear infection, ($p = 0.010$).

Table 2: Distribution of bacterial isolates from patients of ear infection according to age groups and gender.

Studied variables		Bacterial Isolates			
		Positive		Negative	
		No.	%	No.	%
Age groups (years)	≤10	72	42.1	3	30
	11-20	22	12.9	0	0
	21-30	29	16.9	4	40
	31-40	16	9.4	0	0
	41-50	10	5.8	0	0
	≥51	22	12.9	3	30
	Total	171	100	10	100
P-value		<0.001			
Gender	Male	68	39.8	6	60
	Female	103	60.2	4	40
	Total	171	100	10	100
	P-value	0.007			

3.4. Distribution of bacterial isolates according to education level and residence:

Table 4 displays the distribution positive bacterial isolates according to educational background and residence. Positive infections were more frequent among the illiterate group with 74 (43.3%), followed by secondary or higher education 54(31.6%) and primary education 43(25.1%). This distribution was statistically significant ($P = 0.013$). Concerning place of residence, urban residents recorded the highest number of infections with 96 (56.1%), compared with 75 (43.9%) among rural residents, However, this was not statistically significant ($P = 0.108$).

Table 4: Distribution of bacterial isolates from patients of ear infection according to residence and education level.

Studied variables		Bacterial Isolates			
		Positive		Negative	
		No.	%	No.	%
Education level	Illiterate	74	43.3	4	40
	Primary	43	25.1	2	20
	Secondary or higher	54	31.6	4	40
	Total	171	100	10	100
	P-value	0.013			
Residence	Urban	96	56.1	5	50
	Rural	75	43.9	5	50
	Total	171	100	10	100
	P-value	0.108			

3.5. Distribution of bacterial isolates according to behavioral and health-related factors.

This study illustrated the distribution of ear infection cases with positive cultures depending on participants' behavioral and health-related factors. Exposure to water emerged as the most significant risk factor, with infection rates reaching 98.1% among individuals who had recently been exposed to water in the organ, followed by 96.4% among those who practiced regular swimming. In addition, the use of cotton swabs inside the organ (95.6%) and taking antibiotics without a prescription (95.6%) were associated with relatively high infection rates, representing behavioral factors with elevated risk (Table 5).

Conversely, certain behaviors were associated with comparatively lower infection rates. These included drying the organ after swimming or bathing (93.3%) and recent use of medical treatments (91.9%). Frequent use of earphones (93.9%) and immunodeficiency (93.5%) showed only minimal increases in infection prevalence (Table 5).

Table 5: Distribution of bacterial isolates from patients of ear infection according to behavioral and health-related factors.

Risk Factors	Answer option	Total	Bacterial Isolates		P-value
			Positive		
			No.	%	
Suffering from chronic diseases	Yes	31	28	90.3	0.266
	No	150	143	95.3	
Previous ear infection	Yes	64	61	95.3	0.715
	No	117	110	94.0	
Swimming regularly	Yes	55	53	96.4	0.462
	No	126	118	93.7	
Drying the ears after bathing or swimming	Yes	90	84	93.3	0.504
	No	91	87	95.6	
Insert cotton swabs or tools into the ear	Yes	114	109	95.6	0.382
	No	67	62	92.5	
Taking antibiotics without a prescription	Yes	68	65	95.6	0.611
	No	113	106	93.8	
Regular use of hearing aids or ear devices	Yes	49	46	93.9	0.830
	No	132	125	94.7	
Recent water entry into the ear	Yes	107	102	98.1	0.546
	No	74	69	93.3	
Ear cleaning using internal objects	Yes	80	74	92.5	0.301
	No	101	97	96.0	
Recently used ear treatment	Yes	62	57	91.9	0.280
	No	119	114	95.8	
Immune deficiency or long-term use of corticosteroids	Yes	31	29	93.5	0.804
	No	150	142	94.7	

4. Discussion

The present study demonstrated that *P. aeruginosa* (45.6%) and *S. aureus* (23.9%) were the predominant bacterial pathogens isolated from ear infection cases. This finding is consistent with several regional and international studies. The bacteriological profile observed in this study aligns with data from several reports across Yemen, which consistently identify *S. aureus* and *P. aeruginosa* among the predominant etiological agents of ear infections. In Sana'a, Al-Shami *et al.* [13] and Al-Khamesy and Al-Shamahy [10] documented these two pathogens as the most frequent isolates, consistent with findings from Thamar [24], Al-Hodeidah [12], and pediatric cohorts [14], where *S. aureus* isolation rates ranged from 42.9% to 54.6%. Although *S. aureus* generally predominates, localized variations have been reported. In Hadhramout, for example, *S. aureus* accounted for 45% of isolates alongside a 60% prevalence of acute otitis media [11], whereas Al-Khamesy and Al-Shamahy [10] reported a higher prevalence of *P. aeruginosa* (34.95%) than *S. aureus* (14.6%) in Sana'a.

Similar findings have been reported internationally. In Saudi Arabia, Addas *et al.* [25] identified *P. aeruginosa* and *S. aureus* as the dominant pathogens among ear infection cases. In Libya, the most prevalent isolates were *S. aureus* (16.5%) and *Pseudomonas* spp. (14%) [26]. Likewise, in Tanzania, *S. aureus* and *P. aeruginosa* were the most frequently isolated bacteria [16]. In contrast, a study conducted in Ethiopia reported *Proteus* spp. as the most common isolate (27.5%) from ear discharge samples [27]. In addition, a recent systematic review from Ethiopia demonstrated that the prevalence of bacterial ear infections ranged from 28.2% to 98.2%, with a pooled prevalence of 73.39% [28]. These findings indicate considerable geographical and population-related variation in the bacterial distribution of ear infections. Other organisms, including *Enterococcus* spp., *Streptococcus pneumoniae*, *Proteus mirabilis*, and *Klebsiella* spp., were also reported in these studies, although generally at lower frequencies.

The predominance of *P. aeruginosa* and *S. aureus* may be related to their ability to colonize and persist in the ear environment. Moisture particularly favors *P. aeruginosa*, while both organisms possess virulence mechanisms that facilitate adhesion, colonization, persistence, and tissue damage [29-31]. These characteristics may partly explain their frequent isolation across different geographical and clinical settings.

Regarding age distribution, the present study showed that ear infections were most common among patients aged ≤ 10 years (42.1%), followed by those aged 21–30 years (16.9%). Moderate frequencies were observed among individuals aged 11–20 years and ≥ 51 years (12.9%). This finding is consistent with previous national data

from Yemen, where Ben Mohanna and Bahannan [14] reported a remarkably high prevalence among children, particularly those under five years of age (66.6%). Parallel local trends have been documented in Hodeidah and Thamar [12, 24]. Internationally, these results align with epidemiological profiles reported in Ethiopia [6, 32] and Nigeria [33], reinforcing a broader consensus in the literature that identifies children and young adults as the sub-populations bearing the highest burden of ear infections [2, 34].

The higher frequency among children may be related to age-dependent anatomical and immunological characteristics and their greater susceptibility to upper respiratory tract infections [6, 35]. Evidence from a recent systematic review also supports the importance of age-related and other host factors in childhood chronic suppurative otitis media [36]. The relatively high frequency observed among young adults in the present study may reflect differences in exposure patterns and healthcare-seeking behavior.

Ear infections were more common among females (60.2%) than males (39.8%) in the present study. Similar female predominance has been reported in studies from Yemen [11,12], Saudi Arabia, Libya and Iraq [25, 26, 37], whereas higher rates among males have been reported in Yemen [10, 13], Iraq [2], and Ethiopia [27, 34].

These differences may reflect variation in demographic characteristics, healthcare-seeking behavior, sociocultural practices, and study design. Importantly, current evidence does not support a consistent sex-related difference across all forms of ear infection. A systematic review and meta-analysis showed no significant association between sex and the development of chronic suppurative otitis media in children [36]. Therefore, the female predominance observed in the present study should be interpreted cautiously rather than attributed to a specific biological mechanism.

Analysis of educational level showed that ear infections were more prevalent among individuals with lower educational attainment, particularly illiterate participants (43.3%). This finding may be associated with differences in health awareness, ear-care practices, healthcare-seeking behavior, and medication use. Similar associations between educational or socioeconomic factors and ear disease have been reported previously [12, 38]. However, educational level may also reflect broader socioeconomic and behavioral characteristics and should therefore not be considered an independent causal factor without further analysis.

Concerning place of residence, ear infections were more frequent among urban residents (56.1%) than rural residents (43.9%). This observation agrees with previous findings [12, 36]. The difference may be related to variations in population density, environmental

exposure, lifestyle practices, healthcare utilization, and access to medications without prescription in urban settings.

Behavioral and health-related practices were frequently reported among infected participants. Recent water exposure and regular swimming were particularly common, while cotton-swab insertion and unsupervised antibiotic use were also frequently reported. Moisture associated with swimming is a recognized risk factor for otitis externa and may favor the growth of organisms such as *P. aeruginosa* [39, 40]. Cotton-swab use may cause mechanical irritation or trauma to the ear canal and has been identified as an important risk factor for otitis externa in children [41]. These findings support the importance of preventive education regarding excessive water exposure, insertion of objects into the ear canal, and inappropriate antibiotic use.

This study represents the first report to highlight the bacterial etiologies and selected demographic and behavioral factors associated with ear infections in the study region. These empirical data directly contribute to bridging a critical knowledge gap regarding local microbial distribution patterns. Furthermore, the findings establish a vital baseline database for decision-makers, clinicians, and researchers facilitating the optimization of empirical treatment protocols and guiding local stewardship initiatives.

The study was conducted in a specific healthcare setting, which may limit the generalizability of the findings to other populations or geographical areas. Some behavioral information was self-reported and may therefore be subject to recall or reporting bias. Antimicrobial susceptibility results were deliberately not included in the present manuscript because they have been retained for a separate publication specifically addressing the antimicrobial susceptibility patterns of the bacterial isolates.

5. Conclusion:

The present study demonstrated that *P. aeruginosa* was the predominant bacterial isolate among patients with ear infections, with a statistically significant predominance compared with other bacterial isolates ($p < 0.001$). Bacterial infection was significantly more frequent among children under 10 years of age ($p < 0.001$), females ($p = 0.007$), and illiterate individuals ($p = 0.013$). Although higher infection rates were observed among urban residents and individuals with recent water exposure, these associations were not statistically significant ($p = 0.108$ and $p = 0.54$, respectively).

These findings highlight the importance of early microbiological diagnosis and appropriate management of bacterial ear infections, particularly among children and other groups with higher infection rates.

Strengthening health education and preventive ear-care practices may help reduce the occurrence and potential complications of ear infections.

Future research should include larger multicenter studies to further investigate the epidemiological and environmental factors associated with bacterial ear infections and to evaluate the antimicrobial susceptibility patterns of the predominant bacterial isolates, supporting evidence-based antibiotic selection and appropriate treatment strategies.

Abbreviation List:

ENT: Ear, Nose, and Throat.

KIA: Kligler Iron Agar.

SIM: Sulfide Indole Motility.

SPSS: Statistical Package for the Social Sciences.

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مقالة بحثية

عزل وتحديد البكتيريا المسببة لالتهابات الأذن وعوامل الخطر المرتبطة بين المرضى المترددين على عيادات الأنف والأذن والحنجرة في عدن، اليمن

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المُلخَص

تمثل التهابات الأذن مشكلة صحية عامة ومهملة في الدول النامية، تؤثر بشدة على ملايين الأطفال والبالغين، لذا هدفت هذه الدراسة إلى تحديد العوامل البكتيرية المسببة لالتهابات الأذن بين المرضى الذين يراجعون عيادات الأنف والأذن والحنجرة في مدينة عدن، وتحديد عوامل الخطر المرتبطة بالمرضى البكتيرية. تم جمع 181 عينة مسحة من الأذن من أغسطس إلى أكتوبر 2025 وزرعتها على الوسائط المناسبة. وتم تحديد عزلات البكتيريا باستخدام خصائص المستعمرات، وصيغة غرام، والاختبارات الكيميائية الحيوية القياسية. من بين 181 عينة، كانت 171 عينة (94.5%) إيجابية للزرع. وكانت الزائفة الزنجارية *P. aeruginosa* الأكثر شيوعاً بنسبة 45.6%، تليها العنقودية الذهبية *S. aureus* (23.9%)، ثم الإشريكية القولونية *E. coli* (15.2%)، و *Klebsiella spp.* (7.6%)، و *Proteus spp.* (4.7%)، و *Streptococcus spp.* (1.8%)، في حين كانت *Enterobacter spp.* و *Citrobacter spp.* الأقل شيوعاً (0.6% لكل منهما). أعلى معدل إصابة إيجابية كان (42.1%) بين الأطفال ≥ 10 سنوات. وكانت أغلب الإصابات الإيجابية لدى الإناث (60.2%) مقارنة بالذكور (39.8%). كانت الإصابات الإيجابية أكثر شيوعاً بين الفئة الأمية (43.3%) وبين سكان المدن (56.1%). وظهر التعرض للماء كأهم عامل خطر (98.1%). كانت بكتيريا الزائفة الزنجارية *P. aeruginosa* أكثر مسببات الأمراض شيوعاً في التهابات الأذن. يُنصح بالتشخيص المبكر، والعلاج المناسب، وتعزيز التوعية الصحية والوقاية، خاصة للأطفال والمجموعات عالية الخطورة الأخرى.

الكلمات المفتاحية: التهاب الأذن؛ العزلات البكتيرية؛ عوامل الخطر؛ محافظة عدن.

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