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Bacterial Isolates from Otitis Media Infections and their Antibiograms, Hodeidah City, Yemen

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Authors' contributions

This work was carried out in collaboration between all authors. Author MS designed the study, performed the statistical analysis and wrote the protocol. Authors AAK, SB and AIM managed literature searches, managed the analyses of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed to determine the predominant bacterial isolates and their antimicrobial susceptibility patterns from ear discharge among children and adults in Yemen.
Study Design: Prospective cross sectional.
Place and Duration of Study: Department of Medicine and Department of Microbiology, Al-Thawrah and Al-Amal hospitals, Hodeidah city, Yemen, between August 2014 to March 2015.
Methodology: Ear swabs collected from 80 patients with otitis media were inoculated into blood, chocolate, MacConkey agar plates and subjected to several bacteriological tests and analysis. Isolated bacterial pathogens were tested against thirteen antibiotics using standard

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bacteriologic techniques.
Results: The most common isolated bacteria were Gram positive (27=42.9%) of *Staphylococcus aurues* and Gram negative (21=33.3%) of *Pseudomonas aeruginosa*. Gentamycin, Tetracyclin, Ciprofloxacin, and Norfloxacin revealed high level of sensitivity to *Staphylococcus aurues*, whereas Polymyxin-B and Cotrimoxazol to *Pseudomonas aeruginosa*.
Conclusion: *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the predominant pathogens of discharging ear. In the study area, gentamicin and tetracycline would be the drug of choice in *Staphylococcus aureus* isolates, while *Pseudomonas aeruginosa* was highly sensitive topolymyxin-B and cotrimoxazol.

Keywords: Otitis media; bacterial pathogens; resistance pattern.

1. INTRODUCTION

Otitis Media (OM) is the inflammation of the middle ear, which may be caused by bacteria, fungi or viruses. There are three types of otitis media, and these are, acute purulent otitis media, otitis media with effusion and chronic suppurative otitis media [1,2].

Otitis media is a major health problem of children in developing countries with poor socioeconomic status. This is due to the fact that children are more prone to otitis media related to the immaturity of their immune status, the shorter and horizontal nature of Eustachian tubes, frequent exposure to upper respiratory tract infections and malnutrition [3-6].

The significant risk factors in OM include socioeconomic status, cultural, seasonal, and age factors, as well as family history of the middle ear disease. Other risk factors include: environmental tobacco smoke, crowded living conditions, daycare attendance, pacifier use, and bottle feeding in a supine position. Hand washing, breastfeeding, and immunizations offer some protection against otitis media [7-14].

In developing countries, Chronic Suppurative Otitis Media (CSOM) occurs as a complication of acute otitis media with perforation and can be a major health issue. The range of bacterial pathogens associated with CSOM is considerably broader than those seen in acute otitis media (AOM). *Pseudomonas*, *Staphylococcus*, *Proteus*, and *Klebsiella* species are most commonly isolated, and mixed infections are common [15-18].

AOM is the most common indication for the use of antimicrobial agents in children [19-22].

The aims of this study were to isolate and identify the bacterial etiology causing Otitis Media, and to determine their susceptibility pattern to the most common used antibiotics.

2. METHODOLOGY

2.1 Study Population

This cross-sectional prospective study was carried out during the period of August 2014 to March 2015. A total of 80 samples from patients (37 male and 43 female) with otitis media infection were seen at the ENT clinics at Al-Thawrah hospital and Al-Amal hospital, Hodeidah city, Yemen; and that were clinically diagnosed to have otitis media. An informed consent was obtained from each patient to collect an ear discharge by the attending physician and filled a predesigned questionnaire, which included questions regarding age, sex, living place, symptoms, medications, previous OM infection(s), and family history of OM.

2.2 Sample Collection, Handling, Culture and Identification

Ear swap/discharge specimens were aseptically collected. Patient's ear was washed by normal saline (0.85% NaCl). Discharges samples were collected using sterile cotton swabs. All ear swabs/discharge were directly inoculated onto blood, chocolate, MacConkey agar (Hi-media, Mumbai, India). The Blood and MacConkey agar plates were incubated aerobically, while chocolate agar plates were incubated anaerobically under 5% CO2 atmosphere both at 37℃ for 24-48 hours. All positive cultures were identified according to standard microbiology methods, including: Cultural characters, Gram stain, biochemical reactions such as: Coagulase, catalase, citrate media and Kilgler Iron Agar (KIA), urease test, indole test, oxidase and diagnostic tests such as gas production, bacitracin and optichin.

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed for all isolates according to the criteria of the (CLSI) clinical and laboratory standards institute. Bacterial suspension was prepared and was adjusted to a McFarland solution 0.5 and inoculated onto Mueller-Hinton agar (Hi-media, Mumbai, India) using disk diffusion method. The susceptibility pattern of the bacterial pathogens was determined towards the following antimicrobial agents, tetracycline, polymyxine-B, erythromycin, gentamicin, ciprofloxacin, norfloxacin, cotrimoxazole, ceftriaxone, amoxicillin, cefotaxime, cefuroxime, chloramphenicol, and clarithromycin.

2.4 Statistical Analysis

Statistical analysis was done using SPSS software version 15. Chi-square test was employed to compare the proportion of bacterial isolates with patients' age and comparison of antimicrobial resistances. P-value of < 0.05 was considered to indicate statistically significant difference.

3. RESULTS

Table 1 shows the distribution of Otitis Media infection according to demographic characteristics of the studied sample. It was evident that the highest infection was 23(36.5%) at age <10 years, followed by the age category 20-30 years 15(23.8%). Approximately the infections were divided between males and females. Majority of infected sample was collected from urban patients 49 (77.8%). Most of the infected patients were illiterates, which showed highly statistically significant relationship (<0.001).

Table 2 demonstrates the distribution of isolated bacteria from patients with Otitis Media according to gender. It was noticed that the most common bacterial infection caused by *Staphylococcus aureus* bacteria (42.9%) followed by *Pseudomonas aeruginosa* (33.3%). Males were

Table 1. Distribution of otitis media infection according to demographic characteristics

Demographic characteristics		Positive (63)		Negative (17)		Total (80)		X^2	p-value
		No	%	No	%	No	%		
Age	<10	23	36.5	7	41.2	30	37.5	72.5	0.13
	10-	14	22.2	3	17.6	17	21.3		
	20-	15	23.8	6	35.3	21	26.3		
	30-	3	4.8	1	5.9	4	5.0		
	40-	5	7.9	0	0	5	6.3		
	50-	2	3.2	0	0	2	2.5		
	60-70	1	1.6	0	0	1	1.3		
	Total	**63**	**100%**	**17**	**100%**	**80**	**100%**		
Gender	Male	30	47.6	7	41.2	37	46.3	7.3	0.50
	Female	33	52.4	10	58.8	43	53.8		
	Total	**63**	**100%**	**17**	**100%**	**80**	**100%**		
Residence	Urban	49	77.8	12	70.6	61	76.3	5.2	0.74
	Rural	14	22.2	5	29.4	19	23.8		
	Total	**63**	**100%**	**17**	**100%**	**80**	**100%**		
Education	University	11	17.5	5	29.4	16	20	95.7	0.00
	Secondary	9	14.3	3	17.6	12	15		
	Preparatory	3	4.8	0	0	3	3.8		
	Primary	3	4.8	0	0	3	3.8		
	Professional	1	1.6	0	0	1	1.3		
	Illiterate	36	57	9	53	45	56.3		
	Total	**63**	**100%**	**17**	**100%**	**80**	**100%**		
Antibiotic use	Yes	24	38.1	5	29.4	29	36.3	15.3	0.05
	No	39	61.9	12	70.6	51	63.8		
	Total	**63**	**100%**	**17**	**100%**	**80**	**100%**		
Collection site	Left Ear	24	38.1	9	52.9	33	41.3	9.9	0.27
	Right Ear	39	61.9	8	47.1	47	58.8		
	Total	**63**	**100%**	**17**	**100%**	**80**	**100%**		

infected more than females by *Staphylococcus aureus*, while females were infected more by *Pseudomonas aeruginosa.* From the isolated bacteria, females were totally infected more than males.

Table 3 illustrates the distribution of isolated bacteria from patients with Otitis Media. It was clear that Gram negative bacteria were more than Gram positive (57.1% to 42.9%). *Staphylococcus aureus* was the only Gram positive bacteria isolated (42.9%), while the most common Gram negative bacteria was *Pseudomonas aeruginosa* (33.3%).

Table 4 shows the distribution of isolated bacteria from Otitis Media patients according to antimicrobial drugs sensitivity. It was found that the most abundant isolated bacteria was *Staphylococcus aureus* (27 (43%)) which was sensitive mainly to both gentamicin and tetracycline (89% each) followed by both norfloxacin and ciprofloxacin (81% each). *Pseudomonas eruginosa* was the second abundant isolated bacteria (21 (33%)) and was mainly sensitive to polymyxin-B and cotrimoxazol (100% and 86% respectively). Cotrimoxazol, gentamicin, ciprofloxacin, and norfloxacin were the most antimicrobial drugs showed an effect on different isolated bacteria.

4. DISCUSSION

The term ‘‘otitis media’’ covers a wide spectrum of disease, and is used to describe illnesses with predominantly middle ear symptoms (including acute otitis media, otitis media with effusion, and chronic suppurative otitis media [23,24].

In the study area, otitis media is common health problem in children more than adults. Among children, peak-age prevalence was observed in the age group under ten years (<10). This is comparable with previous studies conducted in Thamar [25], Ethiopia [26,27] and Nigeria [28].

Similar to a study was done in Pakistan [29]; the present study indicated that OM is more prevalent in females (53.75%) than males (46.25%). In contrast, the study in Thamar and Nigeria reported that males were affected more than females [25,28].

The present study is comparable to those reported in Ethiopia [26] and Iraq [30], where generally the Gram negative bacteria were dominant isolates (57.1%) of discharging ears compared to Gram positive bacteria (42.9%).

Table 2. Distribution of isolated bacteria from patients with otitis media according to gender

Bacteria	Male	%	Female	%	Total	%
Staphylococcus aureus	16	53.3	11	17.4	27	42.9
Pseudomonas aeruginosa	8	26.7	13	20.6	21	33.3
Proteus vulgaris	1	3.3	3	4.7	4	6.3
Proteus mirabilis	2	6.7	2	3.2	4	6.3
Klebsiella pneumonia	1	1.6	2	3.2	3	4.8
Escherichia coli	1	1.6	0	0.0	1	1.6
Provedicia species	0	0	1	1.6	1	1.6
Salmonella paratyphi	0	0	1	1.6	1	1.6
Other *Salmonellae*	1	1.6	0	0.0	1	1.6
Total	**30**	**47.7**	**33**	**52.3**	**63**	**100**

Table 3. Distribution of isolated bacteria from patients with otitis media

Gram stain	Bacteria	Number	%
Gram positive	*Staphylococcus aureus*	27	42.9
Gram negative	*pseudomonas aeruginosa*	21	33.3
	Proteus vulgaris	4	6.3
	Proteus mirabilis	4	6.3
	Klebsiella pneumonia	3	4.8
	Escherichia coli	1	1.6
	Provedicia species	1	1.6
	Salmonella paratyphi	1	1.6
	Other *Salmonellae*	1	1.6
Total		**63**	**100**

Table 4. Distribution of isolated bacteria from otitis media patients according to sensitivity to antimicrobial drugs

Isolated bacteria		Sensitivity to antimicrobial drugs												
		Tetracycline	**Erythromycin**	**Chloramphnicol**	**Gentamicin**	**Ciprofloxacin**	**Norfloxacin**	**Cotrimoxazol**	**Ceftriaxone**	**Amoxacilin**	**Cefotaxime**	**Cefuroxime**	**Clarithromycin**	**Polymyxin-B**
Staphylococcus aureus (27)		24	1	1	24	22	22	19	3	17	7	5	5	1
Pseudomonas aeruginosa (21)		2	3	4	9	10	9	18	1	2	3	3	4	21
Proteus vulgaris (4)		1	--	1	2	3	3	3	3	1	1	2	1	--
Proteus mirabilis (4)		3	--	--	3	3	3	--	3	--	1	2	--	--
Klebsiella pneumonia (3)		3	--	1	3	2	3	3	--	--	1	1	--	1
Escherichia coli (1)		--	--	--	--	--	--	--	--	--	--	--	1	--
Salmonella paratyphi (1)		--	--	--	--	--	1	--	--	--	--	--	--	--
Other *Salmonellae* (1)		--	--	--	--	1	--	--	1	1	--	--	--	--
Provedicia species (1)		1	--	1	1	1	1	1	1	--	--	--	--	--
Total (63)	**No.**	**34**	**4**	**8**	**42**	**42**	**42**	**44**	**12**	**21**	**13**	**13**	**11**	**23**
	%	**54**	**6**	**13**	**67**	**67**	**67**	**70**	**19**	**33**	**21**	**21**	**17**	**37**

Staphylococcus aureus and *Pseudomonas aeruginosa* were the most dominant isolates, in agreement with studies conducted in Thamar [25], Ethiopia [26] and Basrah [30], and oppose to other studies in India and Nigeria, where *Pseudomonas aeruginosa* was isolated more than *Staphylococcus aureus* [28,31].

The most similar study to this study was that done in Iran in which the most isolated pathogen was *Staphylococcus aureus* followed by *Pseudomonas aeruginosa,* and *Proteus species* [32].

The United States (US) Food and Drug Administration (FDA) approved the use of gentamicin for treating different infectious disorders such as, infection by *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens*, *Citrobacter* spp., Enterobacteriaceae species, or *Pseudomonas* spp.; *Staphylococcus* infectious disease. Furthermore, gentamicin is used widely beyond its FDA-labeled indications such as, *Actinomycotic* infection; and *Staphylococcus saprophyticus* bacteremia with pyelonephritis [33].

In this study *Staphylococcus aureus* revealed a high level of sensitivity to gentamicin, ciprofloxacin, norfloxacin and cotrimoxazol, while a study in Ethiopia showed high level of resistance to amoxicillin, and low resistance to ciprofloxacin, gentamicin and norfloxacin [26].

Recently study showed that gentamicin is used in combination regimens, such as with beta-lactam antibiotics to treat mixed infection and with bacteriophage to treat *Staphylococcus aureus* infections [33].

Previously, cotrimoxazol was used to treat AOM in adults and children caused by different bacterial such as *Streptococcus pneumoniae* or *Haemophilus influenza* [34,35]. In this study, *Pseudomonas aeruginosa* was highly sensitive to cotrimoxazol.

Pseudomonas aeruginosa was the most antibiotic resistance microorganism isolated in otitis media, most of the isolates were resistant to amoxicillin, erythromycin, tetracyclin, and chloramphenicol, which is comparable to a study in Ethiopia [26], however, *Pseudomonas aeruginosa* was highly sensitive to polymixin-B and cotrimoxazol, in contrast to that study in Ethiopia which showed high sensitivity to norfloxacin, ciprofloxacin and gentamicin [26]. *Pseudomonas aeruginosa* was the second bacteria showing sensitive to gentamicin (9 = 41.4%) after *Staphylococcus aereus* (24 = 57.4%). Recent study showed evaluating synergy between gentamicin and marbofloxacin in *Pseudomonas aeruginosa* strains isolated from dogs with otitis externa [36].

5. CONCLUSION

Otitis media was a common health problem of all age groups especially children. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the predominant pathogens of discharging ear. In the study area, gentamicin and tetracycline (89% each) would be the drug of choice in *Staphylococcus aureus* isolates, while *Pseudomonas aeruginosa* was highly sensitive to polymyxin-B and cotrimoxazol (100% and 86% respectively).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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