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Otitis Media in Children: Identification and Antibiotics Sensitivity of Bacterial Pathogens in Ibb City, Yemen

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Abstract
Otitis media (OM) is an inflammation of the middle ear. Clinically, OM presents as acute otitis media (AOM) and if it persists for more than 3 months it is called chronic otitis media (COM). So, the present study was performed to identify the aerobic bacterial pathogens and determine the antibiotics sensitivity in children with OM in Ibb city, Yemen. Ear swabs were collected from 100 children (Male: 53, Female: 47) that suffered to OM, clinically diagnosed by Ear Nose Throat (ENT) specialist doctors. All patients samples were inoculated into different bacteriological media to isolate the bacterial pathogens using standard bacteriologic techniques and the antibiotics sensitivity against pathogenic bacteria were done using standard disc diffusion technique. According physician and clinical diagnosis, our results showed that OM children were COM (70%) more than AOM (30%). Also, our findings indicated that the growth of aerobic gram positive bacteria was 50 (50%) more than gram negative bacteria 47 (47%) and children with non-growth of bacteria was 3 (3%). The most common type of gram positive bacteria was *Streptococcus pneumoniae* (27%), whereas the most common type of gram negative bacteria was *Pseudomonas aeroginosa* (21%). The broad spectrum antibiotic sensitivity against gram positive and gram negative bacteria showed that the most effective and suitable antibiotics were Kanamycin and Amikacin (92%, 91%, respectively), while an effective antibiotic against *S. pneumoniae* was Vancomycin (80%) and an effective antibiotic against *P.aeroginosa* was Carbencillin (89%). In addition, our results showed that breastfeeding prevented OM in (63.3%) of infected children; whereas, passive smoking enhanced OM infection in (58%) ones. It can be concluded that the routine culture and sensitivity test remains the most important way for treating Otitis media and to prevent the risk of empirical therapy and antibiotic resistance to them.
Keywords: Otitis media, Bacterial Pathogens, Antibiotics Sensitivity, Children, Yemen.

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INTRODUCTION

Otitis media (OM) is an inflammation of the middle ear, which may be caused by bacteria, fungi and viruses; it is primarily disease of infants and young children. It can also affect adults and it presents in several forms that are characterized by both the duration of the disease and the type of exudates (Zhang *et al*., 2014; Suhail *et al.,* 2016). OM is a major health problem of children in developing countries. Collectively, the main risk factors of OM in children include: an immaturity of their immune status, the shorter and horizontal nature of Eustachian tubes, frequent exposure to upper respiratory tract infections and malnutrition, ethnicity, genetic factors, gender, allergy, socioeconomic status, cultural, seasonal, family history , smoking environment, crowded living conditions, pacifier use, bottle feeding in a supine position and less hand washing (Lieberthal *et al*., 2013; Qureishi *et al.,* 2014).

There are three types of OM: Acute purulent otitis media (AOM), otitis media with effusion (OME) and chronic suppurative otitis media (CSOM). Chronic Otitis media (COM) is one of the most common infectious diseases in children, also an untreated infection that can travel from the middle ear to the nearby parts of the head, including the brain, causing temporary and permanent hearing impairment and persistent fluid in the middle ear and it can reduce the speech and language development in the children (Holl *et al*., 2015; Wasihun *et al.,* 2015).

The most common bacterial pathogens found in AOM are: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus infleunzae* and *Micrococcus catarrhalis*, but the bacteria in CSOM may be aerobic: *Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus,*

Streptococcus pyogens, Proteus species, Klebsiella species or anaerobic: *Bacteriodes, Peptostreptococcus, Propioni bacterium* (Prakash *et al*., 2013; Hirapure and Pote, 2014; Saranya *et al.*, 2015; Babeker and Elhag, 2016). In judging the consistency of the previous studies, clinical treatment of OM in developing countries have been disappointed, which due to poor antibiotic penetration into middle ear pus, bacterial resistance to commonly available antibiotics and the extensive use of antibiotics that caused the development of multi-drug resistant of the bacterial pathogens (Smith *et al*., 1996; Macfadyen *et al*., 2006; Elemraid *et al.*, 2011).

In Yemen, there are a few studies about bacterial etiologies and its sensitivity to antibiotics which is an essential on developing proper management (Suhail *et al.,* 2016; Al-Alousi and Mayas, 2012; Muftah and Mackenzie, 2015; Mohanna and Bahannan, 2016). Also, other reports showed that the rational antibiotic usage and successful treatment for OM may be reduced the risk factors and its complications (Elemraid *et al.,* 2009; Elemraid *et al*., 2010; Elemraid *et al.*, 2011). Indeed, previous studies are very limited and more new reports are very important to clarify OM as one of the health problems of all Yemeni children in different area. This study was designed to identify the most common aerobic bacterial pathogens that causing OM of infants and young children in Ibb city, Yemen to determine an effective and a suitable antibiotic against these pathogenic bacteria and to correlate OM infections with the main risk factors.

MATERIALS AND METHODS

This case-control study was conducted with one hundred (100) infected children with OM, which admitted to the committee of General Al-Thowra Hospital- Ibb city as: Male: 53; Female: 47, that suffered to OM, the clinical diagnosis of these patients were performed by ENT specialist doctors and thirty (30) children as: (Male:12 (40%); Female: 18 (60%), apparently healthy as normal controls (NC). The patients and healthy children were divided according their ages as: 2-11 months, Group (I); 1-3 years, Group (II); 4-6 years Group (III) and 7-12 years Group (IV). One hundred and thirty (130) sterile cotton swabs were collected from ear discharges of patients and controls after cleaned of the outer ear by normal saline. All swabs were inoculated into: Nutrient, Blood, MacConkey and Simon citrate and kligler iron agars and were incubated aerobically at 37°C for 24 hours. All positive cultures were identified according to standard bacteriological methods, including: cultural characters, gram stain, different biochemical tests such as: oxidase, coagulase, catalase, lactose, urease and indol. The antibiotics sensitivity test of the bacterial pathogens was done by the standard disc diffusion technique (A liquid bacterial culture of different bacterial pathogens inoculated into nutrient agar plate, the antibiotics discs were placed on the surface of plate under sterile condition, incubated for 24 hrs and then antibiotic resistance was determined by inhibition of bacterial growth around discs (zones of inhibition) (Bauer *et al*., 1966; Michael *et al*., 2012; Iqbal *et al.,* 2015; Iqbal *et al.,* 2016). The available antibiotics in our work were: (1) Broad spectrum antibiotics: Chloramphenicol (C), Kanamycin (KM), Amikacin (AK), Gentamycin (GN) and Augmentin(AG); (2) Antibiotics against Gram +ve bacterial growth: Penicillin (PE), Ampicillin (PN), Vancomycin (VAN), Streptomycin (SE) and Clindamycin (CLD); (3) Antibiotics against Gram -ve bacterial growth: Carbencillin (CAR), Ceftizoxime (CZX), Cephalaxin (CPX) and Cefoxitin (CFX).

Statistical analysis

Data obtained was analyzed by using SPSS software version 20, to compare the percentages of bacterial pathogens and antibiotics sensitivity in all infected children.

RESULTS

Our findings showed that among one hundred (100) OM children: 53(53%) were male and 47(47%) were female, but among thirty (30) healthy children: 12(40%) were male and 18 (60%) were female, as shown in Table 1. Age wise prevalence of OM in children ranged as 2-11 months (17%); 1-3 years (32%); 4-6 years (25%) and 7-12 years represented (26%). According to the clinical diagnosis of the patients by the specialist physician: (30%) were AOM (acute otitis media) and (70%) were COM (chronic otitis media). Our results showed that the breastfeeding of Group I and Group 2 may be prevent OM in 31 (63.3%) of infected children in comparison to 18 (36.7%), which not having breastfeeding. Among the infected children, 58% were at the risk of OM living in smoking environment and 42% of infected children live away from smoking. The growth of bacterial pathogens was among 97% of all patients, whereas 23% of healthy individuals showed bacterial growth.

Our findings showed that gram positive bacterial pathogens among infected children were 50%, which include: *Streptococcus pneumoniae* 27(27%), *Staphyllococcus aureus* 15(15%) and *Streptococcus pyogenes* 8(8%); while, gram negative bacterial pathogens were 47%, which include: *Pseudomonas aeroginosa* 21(21%), *Klebsiella pneumoniae* 12(12%), *Proteus mirabilis* 9(9%) and *Escherichia coli* 5(5%); while 3% had no growth as shown in Figure 1.

According to our results 30% were mixed bacterial pathogens, which include: *Staphyllococcus aureus*+ *Streptococcus pyogenes* 8(27%), *Streptococcus pneumoniae*+ *Staphyllococcus aureus* 6(20%), *Pseudomonas aeroginosa*+ *Proteus mirabilis* 5(17%), *Staphyllococcus aureus*+ *Kelbsiella pneumoniae* 4(13%), *Staphyllococcus aureus*+ *Proteus mirabilis* 4(13%), and *Streptococcus pyogenes*+ *Pseudomonas aeroginosa* 3(10%), as shown in Figure 2.

Table 1. Demographic data of otitis media and healthy children in Ibb City, Yemen.

Demographic Data	Children with Otitis media			Healthy children		
Ages and Sexes N (%)	Male	Female	Total	Male	Female	Total
Group I:2-11 Months.	10 (10%)	7 (7%)	17 (17%)	3 (10%)	4 (13.3%)	7 (23.3%)
Group II:1-3 Years.	19 (19%)	13 (13%)	32 (32%)	4 (13.3%)	6 (20.1 %)	10 (33.4%)
Group III:4-6 Years.	16 (16%)	9 (9%)	25 (25%)	3 (10%)	4 (13.3%)	7 (23.3%)
Group IV:7-12 Years	8 (8%)	18 (18%)	26 (26%)	2 (6.7%)	4 (13.3%)	6 (20%)
Total N (%)	53 (53%) **	47 (47%)	100 (100%)	12 (40%)	18 (60%)	30 (100%)
Otitis media diagnosis N(%)						
Acute Otitis media (AOM)	30 (30%)			-		
Chronic Otitis media (COM)	70 (70%)**			-		
Breastfeeding N (%)	49 (49%)			17 (56.7)		
Yes	31 (63.3%)**			10 (58.8%)*		
No	18 (36.7%)			7 (41.2%)		
Passive smoking N(%)						
Yes	58 (58%)**			8 (26.7%)		
No	42 (42%)			22 (73.3%)**		
Bacterial Pathogens Growth N (%) :	Male	Female	Total			
Yes 97 (97%)**	AOM 17(17%)*	AOM 12(12%)	29(29%)	7 (23%)		
	COM 38(38%) *	COM 30(30%)		23 (77%)**		
Total	68(68%)**					
G+ve bacteria	55(55%) **	42(42%)				
G-ve bacteria	50(50%)*					
	47(47%)					
No 3 (3%)						
	AOM 1 (1%)	AOM 0 (0%)	1(1%)			
	COM 0 (0%)	COM 2 (2%)	2(2%)			

*P<0.05 , **P<0.01

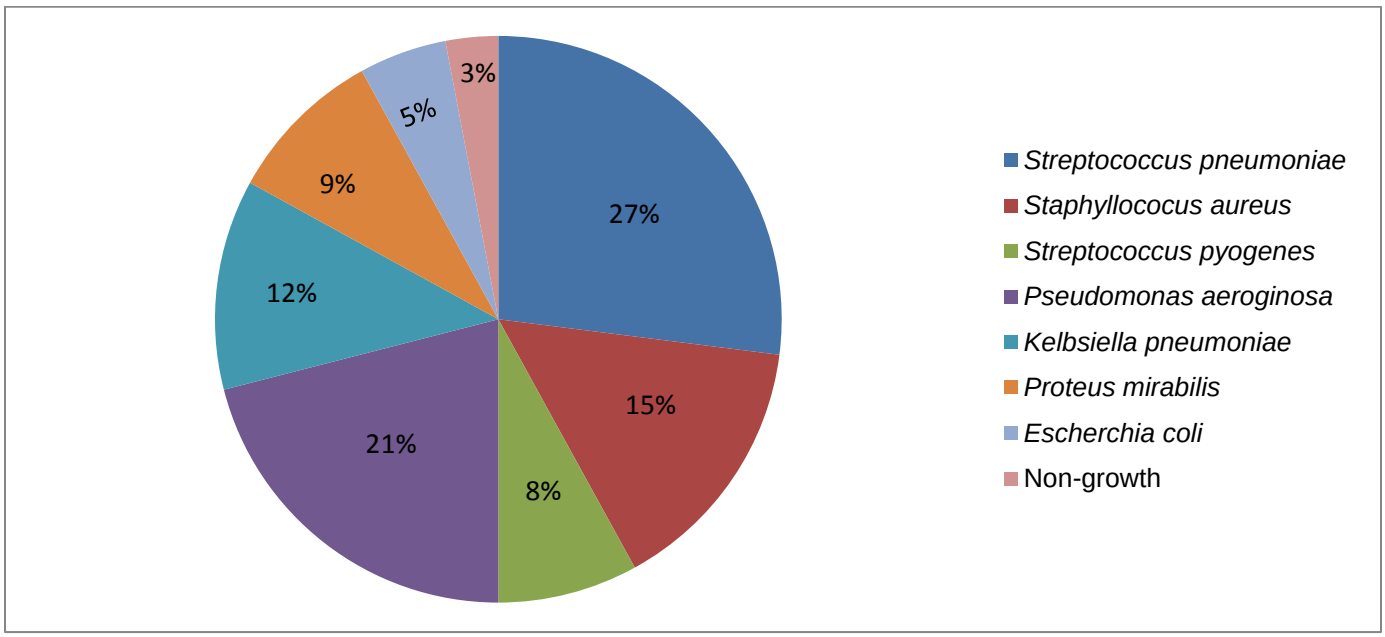

Fig.1. Types of single bacterial pathogens of infected children with OM (%) in Ibb City, Yemen.

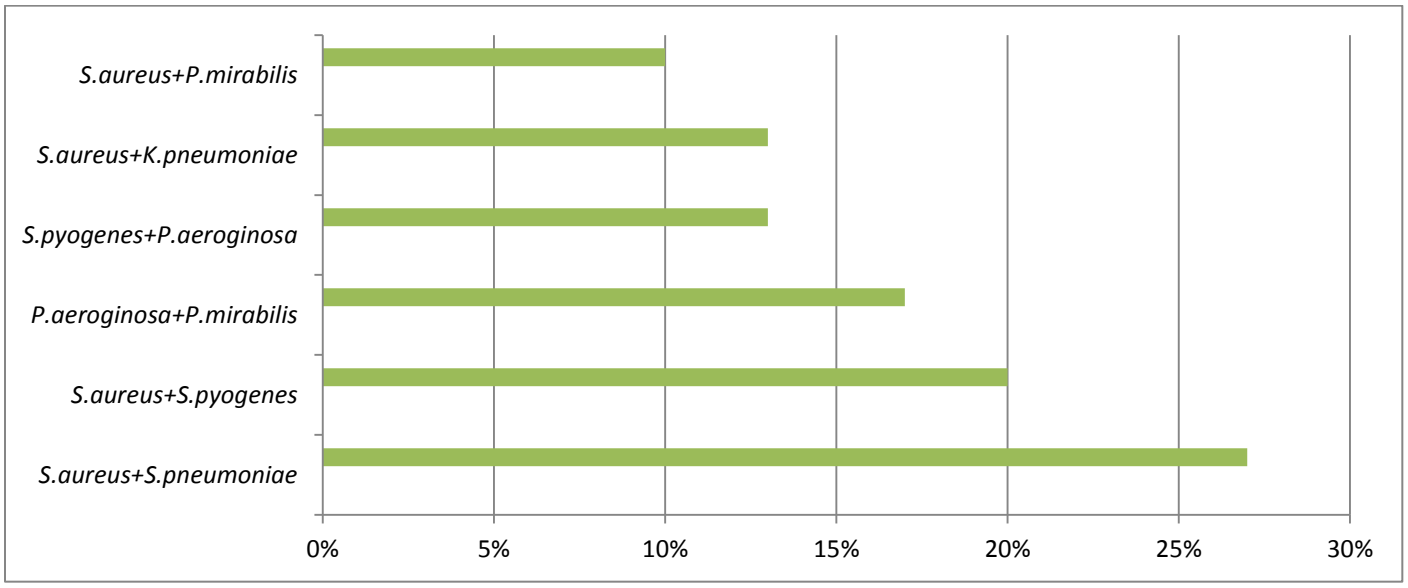

Fig. 2. Types of mixed bacterial pathogens of infected children with OM (%) in Ibb City , Yemen.

Table.2. Antibiotics Sensitivity against bacterial pathogens of OM children in Ibb city ,Yemen.

Types of bacterial pathogens		Antibiotics Sensitivity N (%)													
		Broad Spectrum Antibiotics					Antibiotics against G+ve bacteria					Antibiotics against G-ve bacteria			
G+ve bacteria	*S. Pneumoniae* (27)	C	KM	AK	GN	AG	P	PN	VAN	S	CLD	CAR	CZX	CPX	CFX
		24(89)	24(89)	27(100)	20(74)	14(52)	4(15)	13(48)	22(80)*						
	S. aureus (15)	13(87)	15(100)	13 (87)	8(53)	7(46)	1(7)	7(47)	7(47)		12(82)**				
	S. pyogenes (8)	7(88)	8(100)	6(75)	4(50)	4(50)	1(13)	3(38)	7(88)**	7(88)**					
G-ve bacteria	*P .aeroginosa* (21)	15(71)	21(100)	19(91)	12(57)	11(53)						19(89)**			
	K. Pneumoniae (12)	8(67)	10 (83)	10(83)	8(83)	3(25)							10(84)**		
	P.mirabilis (9)	5(56)	6(67)	9(100)	9(100)	2(22)								8(85)**	
	E.coli (25)	4(80)	5(100)	4(80)	3(60)	2(40)									4(80)**
	Total: 97 (100%)	76(78)	89(92)**	88(91)**	64(66)	43(44)	4(15)	13(48)	7(88)**	7(88)**	12(82)**	19(89)**	10(84)**	8(85)**	4(80)**

C:Chloramphincol ; KM: Kanamy /cin ; AK: Amikacin ; GN: Gentamycin ; AG: Augmentin ; P: Pencillin ; PN: Ampicillin ; VAN: Vancomycin ; S: Streptomycin ; CLD :Clindamycin ; CAR: Carbencillin ; CZX: Ceftizoxme ; CPX: Cephalexin ; CFX: Cefoxitin.*P < 0.05, **P < 0.01.

Finally, the antibiotics sensitivity was determined towards available antibiotics. The results of broad spectrum antibiotics were: Kanamycin (KM) (92%) followed by Amikacin (AK) (91%), Chloramphenicol (C) (78.4%), Gentamycin (GN) (66%) and Augmantin (AG) (44%). While results of antibiotic sensitivity against G+ve bacterial pathogens were: (1) Penicillin (PE): *S.pneumoniae* (15%), *S.aureus* (7%), *S.pyogenes* (13%); (2) Ampicillin (PN): *S.pneumoniae* (48%), *S.aureus* (47%), *S.pyogenes* (38%); (3) Vancomycin (VAN): *S.pneumonia* (80%), *S.aureus* (47%), *S.pyogenes* (88%); (4) Streptomycin (SE): *S.pyogenes* (88%) and (5) Clindamycin (CLD): *S.aureus* (82%). Whereas results of antibiotics sensitivity against G-ve bacterial pathogens were: (1) Carbencillin (CAR): *P.auroginosa* (89%); (2) Ceftizoxime(CZX): *K.pneumoniae* (84%); (3) Cephalaxin(CPX): *P.mirabilis* (85%) and (4): Cefoxitin (CFX) *E.coli* (80%), as shown in Table 2.

DISCUSSION

OM is a potentially serious health problem in Yemeni children and may be complicated by COM, which causes substantial hearing impairment and it carries a risk of permanent hearing loss. Previous gene studies showed that genetic factors of the immune system is considered to be an important prognostic for the risk of OM, which included: TNF-α, IL-6, IL- 10, CD14, IFN-γ, HLA frequencies and properdin deficiency (Rye *et al.,* 2011).

Our finding indicated that the growth of bacteria were significantly (P<0.01) more in male children 55 (55%) than female children 42(42%). These results were similar to previous studies that reported OM children males were affected than females (Al-Alousi and Mayas, 2012; Kawo *et al.,* 2010; Habibu, 2015). While other studies demonstrated that OM is more prevalent in females than males (Suhail *et al.,* 2016; Ahmed *et al.,* 2010**)**. Also, the results showed that the bacterial pathogens were more prevalent in COM children (70%), P<0.01 than AOM children (30%), these results were in agreement with several previous studies which demonstrated that a high prevalence of CSOM among children in developing countries, due to: recurrent upper respiratory tract infection, overcrowding, poor hygiene, swimming in local pools and parents with low education about this infection (Muftah and Mackenzie, 2015). In addition, these results are in agreement with many authors (Muftah and Mackenzie, 2015; Bluestone, 1998; Roland and Stroman, 2002; Yerhoeff *et al.,* 2005; Van der Veen *et al.,* 2006; Kong and Coates, 2009; Jacoby *et al.,* 2011), whom illustrated that the COM is widely seen in children, usually followed by an infection with AOM, which due to a direct passage of pathogens through contaminated water that enter the ear during bathing or swimming, then the pathogens enter tympanic membrane through the Eustachian tube into the middle ear, which resulted CSOM. The CSOM caused a hidden disability, adversely affects cognition and school performance, delaying the development of speech and increasing the risk of life threatening complications, however, the perforation of the tympanic membrane could result from other causes, including trauma.

Our results showed that the breastfeeding of infected children may prevent OM in 31(63.3%) in comparison to 18 (36.7%) non-breastfeeding. This finding is in agreement with others (Uhari *et al.,* 1996; Labbok *et al.,* 2004; Sabirov *et al*., 2009), who indicated that breastfeeding have anti-microbial, anti-inflammatory and immunomodulatory agents that contribute to an optimal immune system in OM children and they also reported that breastfeeding could decrease a risk of AOM in children. While, other authors demonstrated that patients with COM and recurrent chronic otitis media (ROM) did not show a significant difference within the healthy breastfeeding children (Mew and Meredith, 1992; McNiel *et al.,* 2010). Our results showed that among the infected children, 58% were at the risk of OM living in smoking environment and 42% of infected children live away from smoking. These results were in agreement with others, whom reported that environmental smoking (Parental smoking , Second-hand smoking**"** nicotine and other smoking products**"** has been associated with AOM, ROM and COM, they suggested that exposure to smoke could enhance the pathogenic microorganism invasion to the middle ear and impaired the mucociliary function of the Eustachian tube (ET), which resulting in blockage of the nasopharyngeal airway and these microorganism adherence to the epithelial cell surface and depression of local immune system (Fukuma *et al.,* 1986; Holt, 1987; Kum *et al.,* 2006).

Our findings showed a significant difference (P<0.05) between the growth of gram positive and gram negative bacterial pathogens, which is in agreement with many other reports (Al-Alousi and Mayas, 2012 ; Ahcloy *et al.,* 2002; Roland and Stroman, 2002; Baghza, 2007). These results are disagreement with other previous reports that indicated that G-ve bacteria in OM were more dominant when compared with G+ve bacteria (Al-Abbasi *et al.,* 2010; Abera and Kibret, 2011; Prakash *et al.,* 2013). Our findings about the presence of aerobic G+ve bacterial pathogens and mixed bacterial pathogens are in agreement with other studies (Suhail *et al.,* 2016; Saranya *et al.,* 2015; Al-Alousi and Mayas, 2012; Mohanna and Bahannan, 2016; Prakash *et al.,* 2013; Elmanama *et al.,* 2014; Wasihun and Zemene, 2015), whom demonstrated that most common aerobic bacterial pathogens that caused OM in children are *Streptococcus Pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Streptococcus pyogenes, Klebsiella pneumoniae and Escherichia coli* for isolated and mixed infections .

Finally, results of antibiotic sensitivity are in agreement with other reports (Suhail *et al.,* 2016; Saranya *et al*.;2015; Babeker and Elhag, 2016; Al-Alousi and Mayas, 2012; Mohanna and Bahannan, 2016; Habibu, 2015; Elmanama *et al.,* 2014; Al-Saimary *et al.,* 2010; Sahu *et al.,* 2014), who demonstrated that the increasing

emergence of antibiotic resistance of bacterial pathogens during OM, because of geographical differences, population variances, local antibiotics prescribing, indiscriminate and random antibiotic use, irregular follow-up of patients. In addition, the high rate of OM in the developing countries could be due to the socio-cultural behavior of the children, the low immune status, overcrowding, poor sanitation and inadequate medical facilities. Finally, our opinion in our country "Yemen" the incidence of antibiotic resistant to bacterial pathogens in infected children with OM might be due to the overuse and misuse of antibiotics, which lead to the death of sensitive strains and leaving resistant strains to survive, multiply and may be infect other children.

CONCLUSION

Our results demonstrated that the routine culture and sensitivity test remains the most important way for treating AOM and COM due to determine types of bacterial pathogens and to prevent the risk of empirical therapy and antibiotics resistance to them. Also, our findings indicated that the most cases of OM are COM and the most G+ve bacterial pathogen was *S.pneumonia*e and G-ve bacterial pathogens was *P. aeroginosa* and the most effective broad spectrum antibiotics were Kanamycin and Amikacin, while, an effective antibiotic against *S. pneumonia*e was Vancomycin and the effective antibiotic against *P.aeroginosa* was Carbencillin.

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CONFLICT OF INTEREST

There is no conflict of interests regarding the publication of this paper.

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