

International Journal of Advanced Research in Biological Sciences

ISSN : 2348-8069

www.ijarbs.com

Research Article



Study of susceptibility patterns of clinical *Staphylococcus aureus* isolated from patients with Otitis media in Missan

Hateet, R.R.

Department of Biology, College of Sciences, University of Missan, Iraq.

*Corresponding author: seseadh04@mans.edu.eg/seseadh@yahoo.com

Abstract

The present study was designed to the prevalence of multidrug resistant *Staphylococcus aureus*, which was done in Missan, Iraq, during the period between September 2013 and August 2014. A total of eightieth Ear swab were collected from eightieth patients (47 male and 33 female) with chronic and acute suppurative otitis media (CSOM, ASOM) with or without discharges. Fifty seven 57.31% isolates diagnosed as *Staphylococcal spp* and Fortieth 40.0% as *S. aureus* isolates from (26 male and 16 female). *S. aureus* isolates tested for antibiotic susceptibility high antibiotic resistance to Ampicillin, Carbenicillin, Amoxicillin, Nalidixic acid, and Ceftriaxone, mid resistance to Teicoplanin and Co.trimoxazol and sensitive to Tetracycline, Erythromycin. *S.aureus* isolates showed multiple antibiotic resistance, Such that, four isolates resisted six types of antibiotics, six isolates were resisted seven types of antibiotics, three isolates resisted eight types of antibiotics. Five isolates resisted nine types of antibiotics. three isolates resisted ten types of antibiotics. three isolates resisted eleven types of antibiotics. three isolates resisted thirteen types of antibiotics. three isolates resisted fourteen types of antibiotics. two isolate resisted fifteen types of antibiotics. The MAR index of the isolates ranged between 0.3 and 0.75.

Keywords: *Staphylococcus aureus*, otitis media, antibiotic resistance.

Introduction

Otitis media in general, is an inflammation of the middle ear. The most common bacterial pathogens in Otitis media are *Streptococcus pneumoniae*, *Haemophilus influenza* and *Moraxella catarrhalis*, and the other pathogens responsible for Otitis media are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp*, *Pseudomonas aeruginosa*, and *Proteus spp* (Al-Marzoqiet al., 2013). Literature reported that the geographical area and respiratory infections may affect the type of Otitis media pathogens (Al-Marzoqiet al., 2013). Chronic Supportive Otitis Media (CSOM) is defined as persistent or intermittent infected discharge of more than three months duration through the perforated or non intact tympanic membrane that often results in partial or total loss of the tympanic membrane (Shrestha et al., 2011, Prakash et al., 2013), caused

by dysfunction of the Eustachian tube and bacterial infections (Chang et al., 2011). There are reports describing bacteriologic distributions of the middle ear (ME), mastoid and external auditory canal in normal and CSOM patients (Albert et al., 2005; Yeo et al., 2007; Chang et al., 2011). Some studies have acknowledged the value of the nasopharyngeal bacterial reservoir as the etiology of acute otitis media (Syrjanen et al., 2006). The genus *Staphylococcus* has at least 40 species. The three most frequently encountered species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. *S. aureus* is coagulase-positive, which differentiates it from the other species. *S. aureus* is a major pathogen for humans. Almost every person will have some type of *S. aureus* infection during a life time, ranging in

severity from food poisoning or minor skin infections to severe life-threatening infections (Brook *et al*, 2011). *Staphylococcus aureus* regarded as one of the most virulent microbial pathogens which causes nosocomial and community acquired infections and frequently causes bloodstream infections, skin and soft tissue infections, pneumonia and post-operative wound infections (Randrianirina *et al.*, 2007). Nosocomial infections can be caused by a wide variety of pathogens, *S. aureus* is one of the common of both endemic and epidemic infections acquired in hospitals which result in substantial morbidity and mortality (Geffers *et al.*, 2004). *S. aureus* was the commonest microorganism cultured in otitis media (Iseh *et al.*, 2004), causing 50% or more of hospital-acquired *S. aureus* infections in several republics (Wolter *et al.*, 2008). Nasal carriage of *S. aureus* has been identified as a risk factor for community-acquired as well as nosocomial infections (Cole *et al*, 2001). Infections by *S. aureus* are often difficult to treat because of frequency of multiple antibiotic resistance of strains (Alghaithy *et al*, 2000). Multidrug-resistant strains of staphylococci have been reported with increasing frequency worldwide, including isolates that are resistant to methicillin, lincosamides, macrolides, aminoglycosides, fluoroquinolones, or combinations of these antibiotics (Von *et al*, 2001).

Materials and Methods

Study design, area and period

A retrospective study was conducted from September, 2013 to August, 2014 at Sadler Teaching Hospital, Missan city, Southern Iraq.

Culture and identification

A total of eightieth (80) patients with otitis media were included in this study, The diagnosis of otitis media was carried out under the supervision of the specialists of ENT. Microbiological investigation includes (culture and identification of causative agents' antibiotic sensitivity. Collected swabs were taken under sterile condition and transferred immediately to the laboratory by brain heart broth tubes, Primary isolation on selective media to *Staphylococcus* (manitol salt agar) at 37°C for 24 - 48 hr. then the identification and the biochemical characterization were carried out according to standard routine techniques (Cole *et al*, 2001). Well isolated colonies were picked up and stored in nutrient agar slant at 4°C. The pure culture was made by picking single colonies from the stored isolation cultures.

Media and culture conditions:

Blood agar, Nutrient agar, Nutrient broth, Manitol salt agar, Muller Hinton agar, (Himedia, India) Brain-Heart infusion broth, Urease base agar. sterilized by autoclave 121 under 15 lbs pressure for 15 min. Bacterial culture was done at 37°C throughout the experiment. and use antibiotics discs (Table 1).

Table 1: Antibiotics discs, (Oxoid).

Antibiotics	Concentration	Antibiotics	Concentration	Antibiotics	Concentration
Ampicillin	10 mg	Neomycin	30 mg	Clindamycin	2 mg
Amoxicillin	25 mg	Nalidixic acid	30 mg	Oxacillin	1 mg
Carbincillin	100 mg	Erythromycin	15 mg	Kanamycin	30 mg
Norfloxacin	10 mg	Tetracycline	30mg	Vancomycin	30 mg
Ceftriaxon	30 mg	Ciprofloxacin	5 mg	Teicoplanin	30 mg
Cephalexin	30 mg	Gentamicin	10 mg	Co.trimoxazol	30 mg
Tobramycin	10 mg	Streptomycin	10 mg		

Results

Isolation and Identification

A total of 80 aural swab collected from patients with otitis media, 47 bacterial isolates were grown on

mannitol agar plates subjected to hemolytic test on blood agar, gram staining, and catalase test in an attempt to screen the β -hemolytic, gram positive, and catalase positive strains. and according to their growth morphology and biochemical reactions patterns such as coagulase, DNase and β -Galactosidase tests.

in respect to cultural characteristics and colony morphology it on blood agar the colonies are circular, on microscopic observation it was revealed that the cells were arranged in pair or short chain and clusters, golden yellow on nutrient agar, the isolates were coagulase, DNase and β -Galactosidase positive. was found only 32 (40%) isolates were identified as *Staphylococcus aureus* and according to their growth morphology and biochemical reactions patterns such

as β -Galactosidase tests and coagulase, DNase. in admiration to cultural characteristics and colony morphology it was establish that on blood agar the colonies are circular, golden yellow on nutrient agar, on microscopic examination it was exposed that the cells were arranged in pair or short chain and clusters, the isolates were β -Galactosidase and coagulase, DNase positive (Table 2).

Table 2: Frequency of *S. aureus*, CONS and other bacteria from otitis media.

No. of The samples	No. of Staphylococcal isolates			Others(%)
	Total (%)	<i>S aureus</i> (%)	CONS (%)	
80	47 57.31	32 40.0	15 18.75	33 41.25

Antibiotic susceptibility test

Antibiotic susceptibility patterns by antibiotic disc diffusion method all the 32 *Staphylococcus aureus* isolates were tested in vitro. All isolates showed multiple antibiotic resistances to the antibiotics tested,. All isolates (100%) were resistant to Ampicillin, Amoxicillin and, Carbenicillin while Ceftriaxone showed resistance (75%), Nalidixic acid

(87%), Tobramycin, Clindamycin (56.25%), Kanamycin, Vancomycin (50%), Oxacillin (46.87%), Tetracycline (37.5%), Gentamicin (34.37%), Erythromycin, Neomycin, Teicoplanin (31.25%), Cephalexin, (25.0%) , Co.trimoxazole (18.75%), and there was no resistance found to Norfloxacin, Ciprofloxacin and Rifampicin. These antibiotics the most effective against *S. aureus* isolated from acute otitis media the results showed in table (3) .

Table 3: Susceptibility of clinical isolates of *S.aureus* to 20 different antibiotics.

Antibiotic	Abbreviative	Resistans	%	Intermediate	%	Sensitive	%
Ampicillin	AM	32	100	0	0.0	0	0.0
Amoxicillin	AX	32	100	0	0.0	0	0.062
Carbincillin	PY	32	100	0	0.0	0	0.0
Norfloxacin	NOR	0	0.0	16	50.0	16	50
Ceftriaxon	CRO	24	75.0	6	8.75	2	6.25
Cephalexin	CL	8	25.0	10	31.25	14	43.75
Tobramycin	TOB	18	56.25	12	37.5	2	26.25
Nalidixic acid	NA	28	87.5	0	0.0	4	12.5
Erythromycin	E	10	31.25	6	18.75	17	53.12
Tetracycline	TE	12	73.5	0	0.0	20	62.5
Ciprofloxacin	CIP	0	0.0	16	50.0	16	50.0
Neomycin	N	10	31.25	10	31.25	12	37.5
Gentamicin	CN	11	34.37	0	0.0	21	65.62
Rifampicin	RA	0	0.0	3	39.37	29	90.0
Clindamycin	DA	18	56.25	0	0.0	14	43.76
Oxacillin	OX	15	46.87	10	31.25	7	21.87
Kanamycin	K	16	50.0	6	18.75	10	31.25
Vancomycin	VA	16	50.0	8	25.0	8	25.0
Teicoplanin	TEC	10	31.25	21	65.62	1	3.12
Co.trimoxazol	COT	6	18.75	16	50.0	10	31,25

Multiple antibiotic resistance

All *Staphylococcus aureus* isolates showed multiple antibiotic resistance. Such that, four isolates resisted six types of antibiotics, Five isolates resisted seven types of antibiotics, three isolates resisted eight types of antibiotics, Five isolates resisted nine types of

antibiotics ,three isolates resisted ten types of antibiotics ,three isolates resisted ten types of antibiotics, three isolates resisted eleven types of antibiotics ,three isolates resisted thirteen types of antibiotics .three isolates resisted fourteen types of antibiotics and two isolate was resist fifteen types of antibiotics.(table 4) and (Table 5)

Table 4: Antibigram patterns to multiple antibiotic resistance isolates.

multiplicity	Number of isolates	patterns of Antibiotic resistance
6	2	AM ,AX,PY,CRO,NA, TE
6	2	AM ,AX, PY,CRO, NA,TOB
7	5	AM , AX, PY,NA,CRO,K,TE
7	1	AM ,AX, , PY NA,CRO,TE,E
8	1	AM , AX, PY, CRO, NA, TOB,E, OX
8	2	AM , AX , PY, CRO ,NA, TE , E ,OX
9	1	AM , AX , PY,CRO,NA, E,OX , TOB, CL
9	4	AM , AX , PY, CRO , NA , VA , DA,CL, TOB
10	3	AM , AX , PY, CRO, NA, VA, CN ,OX TOB, DA
11	3	AM , AX , PY, COT ,N, K , TEC, TOB, DA, OX, VA
13	2	AM , AX , PY, NA ,N, TEC, K ,CN,TOB,OX , DA, VA
13	1	AM , AX , PY,CRO,CN, TOB , TEC, K ,DA,OX, VA
14	3	AM , AX , PY, NA ,N,CN ,TOB, CL,K , E , DA, VA, COT, TEC
15	2	AM,AX,PY,CRO,NA,N,CL,TOB, TE,E,K,CN, TEC,DA,OX

Table 5: Multiple antibiotic resistance index.(MAR Index).

No. of isolates	4	6	3	5	3	3	3	3	2
(%)	12.5	18.7	9.3	15.6	9.3	9.3	9.3	9.3	6.2
No. of antibiotic	6	7	8	9	10	11	13	14	15
MAR Index	0.3	0.35	0.4	0.45	0.5	0.55	0.65	0.7	0.75

Discussion

In the study area, ear discharge cultures were one of the most frequently requested clinical specimen for culture and antimicrobial susceptibility testes. This indicated that otitis media is a common health problem (Hirapure and Pote, 2014). The results of isolation of *Staphylococcus aureus* isolates from patients with acute otitis media in this study was 32 isolate (40%). *Staphylococcus aureus* was the most common agent

in patients with otitis media and not approved , The results determined with The studies completed by (Shetty AK *et al.*, 2014; RaghuKumar *et al.*, 2014). Pathogen city of *Staphylococcus aureus* in acute otitis media are attributable to virulence factors such as coagulase and hemolysin produced by the organisms and the occurrence of this virulence factors in *Staphylococcus aureus* is in conformity with the reports of (Akinjogunla, *et al.*, 2014). in India, Saini reported that *S. aureus* was the commonest isolate in

pediatric patients (Goya *et al.*,2009). Thus, this study presents antibiogram trends in otitis media. *S. aureus* showed high resistant rate. The activities of antibiotics against *Staphylococcus aureus* that were isolated from acute otitis media patients in general Missan Hospital showed the varied levels of multiple antibiotics resistance. The exceedingly increases and emergence of multidrug resistance pathogens in the developing countries can be attributed to the random use of antibiotics, behavioral past history complex socio-economic, behavioral past history and the distribution of drug-resistant pathogens in human medicine (Yang *et al.*,2009). Antibiotic resistance of pathogens typically causative of acute otitis media continues to increase as the appearance of multi-drug resistant strains especially *Staphylococcus aureus* complicate the organization of acute otitis media and increase the danger for treatment failure (Akinjogunla *et al.*,2010). This study revealed that *S. aureus* resistanted the commonly prescribed antimicrobial agents such as Ampicillin ,Carbincillin, Amoxicillin, Nalidixic acid and Ceftriaxon .The results of this study established that the majority of *Staphylococcus* strains that were isolated from otitis media patients showed a high level of sensitivity to Rifampicin (90.62%), Tetracycline (62.5%), and Erythromycin (53.12%).The development of moderate susceptibility to Ciprofloxacin and Tobramycin this indicates that they are not being abused or commonly prescribed This study revealed that *S.aureus* resistanted the commonly prescribed antimicrobial agents such as Ampicillin ,Carbincillin, Amoxicillin, Nalidixic acid and Ceftriaxon. This study results agree with (Egbe *et al.*,2010 ; Lamido *et al.*,2011), therefore it is not rational to prescribe these agents to patients with otitis media caused by CPSA-induced infection in the studied area without first transport out antibiotic sensitivity test on the isolate (Oyeleke *et al.*,2009).

Conclusion

This study exposed that otitis media in patients is highly prevalent caused by *S. aureus*, Most of the isolates showed high levels of antimicrobial resistance to commonly prescribed antimicrobial .and there is the prevalence of antibiotic resistant strains which make a problem in treatment otitis media .Since otitis media is a nosocomial and community acquired infection, it is recommended that congestion in the health institution should be avoided to reduce the spread of the infection within the hospital. *S. aureus* isolates are sensitive to

Rifampicin by disc diffusion method, High percent of isolated *S. aureus* found to be resistant to Ampicillin, Amoxicillin and Carbincillin that may be due to the irrational use of this antibiotic.

References

- Akinjogunla, O. J. and Enabulele,I.O.,(2010) Virulence Factors, Plasmid Profilin and Curing analysis of Multi-drug Resistant *Staphylococcus aureus* and Coagulase negative *Staphylococcus* spp. isolated from Patients with Acute Otitis Media. J.A. Sci.,6(11):1022-1033.
- Akinjogunla, O. J., Ajayi, A.O. and Ekeh, N.O.(2014) Virulence factors and Antibiotic Resistant *Staphylococcus* spp from the Anterior Nares of Apparently Healthy Undergraduate Students in Uyo. J. Am. Sci. 2(11):158-180.
- Albert R. R, Job A, Kuruvilla G, Joseph R, Brahmadathan K. N,John A. (2005). Outcome of bacterial culture from mastoid granulations: is it relevant in chronic ear disease? J Laryngol Otol. 119(10):774–778.
- Alghaithy, A.A.; Bilal, N.E.; Gedebo, M. and Weily, A.H. (2000). Nasal carriage and antibiotic resistance of *Staphylococcus aureus* isolates from hospital and non-hospital personnel in Abha, Saudi Arabia. Trans.Roy.Soc.Trop.Med.Hyg. ,94: 504-507.
- Al-Marzoqi A. H., Hayder S. h., Al-Janabi O., Hussein J. H., Al Tae Z. M., and Yheea S. K. (2013). Otitis Media; Etiology and Antibiotics Susceptibility among Children under Ten Years Old in Hillah city, Iraq. Journal of Natural Sciences Research. 3 (3): ISSN 2224-3186.
- Brook, I. (2011). The Impact of Smoking on Oral and Nasopharyngeal Bacterial Flora. Journal of Dental Research 90 (6): 704-710.
- Chang J, Lee S.H, Choi J, Im G. J, Jung H. H. (2011). Nasopharynx as a Microbiologic Reservoir in Chronic Suppurative Otitis Preliminary Study. Clinical and Experimental Otorhinolaryngology 4(3): 122-125.
- Cole, A.M.; Tahk, S.; Oren, A.; Yoshioka, D. and Kim,J. (2001). Determinants of *S.aureus* nasal carriage. Clin.Diag.Lab.Immunol., 8(6): 1064-1069.
- Egbe, C. ;Mordi, R. ;Omoriege, R.,and Enabulele, O.,(2010).Prevalence of Otitis Media in Okada

- Community, Edo State, Nigeria Macedonian Journal of Medical Sciences., 3(3):299-302.
- Geffers,C.; Zuschneid,I.; Sohr,D.; Ruden,H.and Gastmeier, P. (2004). Microbiological isolates associated with nosocomial infections in intensive care units. *Anesthesiol-Intensiv Med-Notfallmedschmerzther*, 39 (1) : 15-19.
- Goyal R, Aher A, De S, Kumar A. (2009)Chronic suppurative otitis media – A linico-Microbiological study. *Indian J Otol*;15:18-22.
- Hirapure, P.V and Pote M.K.(2014) Microbial Profile and Antibigrams of Active Patients of Chronic Suppurative Otitis Media in Latur, Maharashtra, India. *Int. Res. J. Medical Sci* .Vol. 2(5), 6-9.
- Iseh, K.R. and Adegbite, T. (2004). Acute suppurative otitis media : A clinical profile Sokoto, Nigeria. *Annals Med. J.*, 4: 164-166.
- Lamido, T. Z.; Ibrahim A. R.; Linda C. O.,and Fatima L. A.,(2011). Coagulase positive Staphylococcal-induced Otitis media in the General Hospital Maiduguri, Nigeria and Antibiotic susceptibility patterns of the Aetiological agent. 3(4):65-69.
- Oyeleke, S.B. (2009). Screening for bacteria agents responsible for otitis media and their antibiogram. *J.Afri.Micr.*, 3(5):249-252 .
- palate: microorganism etiology and susceptibilities, *Cleft Palate Craniofac J*, 46(5), 461-467.
- Prakash R, Juyal D, Negi V, Pal S, Adekhandi S, Sharma , Sharm N (2013). Microbiology of chronic suppurative otitis media in a tertiary care setup of Uttarakhand state. *J. India*; 5(4): 282-287.
- Raghu Kumar,KG., Navya,S and Basavarajappa, KG(2014) A Study of Bacterial Profile and Antibiotic Susceptibility Pattern of Chronic Suppurative Otitis Media among Patients attending a Tertiary Care Centre, Davangere. *J. App. Med. Sci*; 2(5B):1606-1612.
- Randrianiriana, F.,J.L.Soaes, J.F.Carod,E.Rastima,V. Thonnier,P. Combe,P. Grosjean and A.J.Talarmin, (2007). Antimicrobial resistance among uropathogens that cause community acquired urinary tract infections in Antananarivo, Madagascar *Antimicrob Chemother*,59(2):309-312.
- Shetty AK, Shetty A(2014) Aerobic bacteriological profile and their antibiotic susceptibility in Chronic Suppurative Otitis Media in patients from Mangalore, Karnataka State. *J Acad Clin Microbiol*;16(1):3-7.
- Shrestha B. L., Amatya R. C., Shrestha I., Ghosh I. (2011).Microbiological profile of chronic suppurative otitis media. *Nepal. J.of ENT Head & Neck Surg*. 2 (2).
- Syrjanen R. K., Herva E. E., Makela P H., Puhakka H. J., Auranen K. J., Takala A. K. (2006). The value of nasopharyngeal culture in predicting the etiology of acute otitis media in children less than two years of age. *Pediatr Infect Dis J*. 25(11):1032-1036.
- Von Eiff, C.; Becker, K.; Machka, K.; Stammer, H. and Peters, G. (2001).Nasal carriage as a source of *S. aureus*bacteria.*N.Engl.J.Med*. 344(1): 11-16.
- Wolter, D.J.; Chatterjee, A.; Varman, M.; Georing, R.V. (2008). Isolation and Characterization of an Epidermic Methicillin-resistant *Staphylococcus aureus* variant in the central United States *J. Clin.Microb*.46: 3548- 3549.
- Yang,j.a.; kim,j.y. and yoon,y.k., (2009). Epidemiological and genetic characterization of methicillin-resistant *Staphylococcus aureus* isolates from the ear discharge of outpatients with chronic otitis media.*korea med.science*,23:762-6.
- Yeo, S. G., Park D. C, Hong S. M., Cha C. I. and Kim M. G. (2007). Bacteriology of chronic suppurative otitis media: a multicenter study. *Acta Otolaryngoal*. 127, (10): 1062-1067