ORIGINAL ARTICLE

Identification and Antibiotic Susceptibility of Pseudomonas Isolates from Otitis Externa

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ABSTRACT

Background: Otitis externa is an inflammation of the ear canal, which may be caused by bacteria especially pseudomonas. Extension of the otitis media can result in osteomyelitis and cranial nerve palsies, possibly lead to a brain infection.

Aim: To identify and antibiotic susceptibility of pseudomonas isolates from samples taken from the ears of patients with otitis externa.

Methods: Total 30 ear samples were tested to isolate *Ps. aeruginosa*. For identification of *Ps. aeruginosa* biochemical tests like oxidase test and catalase test, sugar (lactose) fermentation test etc were done. MacConkey's agar plate was used for the growth of pseudomonas. Isolates of pseudomonas areuginosa were tested for antipseudomonal antibiotics including Gentamycin, Ciprofloxacin and ampicillin. After 24 hrs the diameters of inhibition zone were measured results were recorded as "sensitive" and "resistant".

Results: Frequency and percentage of *pseudomonas* in 30 samples of ear swab was 75%.Sensitivity to ciprofloxacin was 70% and resistance was 29.41% against *pseudomoanas*. Sensitivity to gentamicin was17.64% and resistance was 82.35% against *pseudomoanas.*On the other hand sensitivity to ampiclillin was11.76% and resistance was 88.23% against *pseudomoanas*.

Conclusion: Ciprofloxacin has been stated to be the most effective drug for the treatment of P. aeruginosa infections. Furthermore, the high rate of resistance to gentamicin and ampicillin recorded in this study appears to confirm the previous suggestions that these three drugs should no longer be taken for the treatment of otitis externa.

Keywords: *Pseudomoanas*, Ciprofloxacin, Gentamicin and Ampicillin.

INTRODUCTION

Sensorineural hearing loss is considered an intractable disease. It includes age-related hearing loss, noise-induced hearing loss, hearing loss due to otitis externa etc.

Otitis externa also called swimmer's ear is an inflammation of the outer ear and ear canal. Factors responsible to develop otitis externa are the presence of bacteria that can infect the skin and injury of the skin of the ear canal that allows infection to occur. Extension of the otitis media can result in osteomyelitis and even cranial nerve palsies, which may lead to a CNS infection[1].

The acute form of otitis externa is caused primarily by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Acute otitis externa shows rapid onset of ear canal inflammation, resulting in itching, canal edema, canal erythema and often occurs following swimming or minor trauma. The prevalence rate of acute form is 1% annually..*P. aeruginosa* accounts for 10.1% of all acquired infections including ear infection. The bacteria are found widely in the environment, such as in soil, water, and plants, inadequately chlorinated water of hot tubs and swimming pools. Very mild illnesses like skin rashes and ear infections have been reported in the healthy individuals. Symptoms may include: swelling, ear-ache, itching inside the ear, discharge from the ear and difficult hearing[3].

Otitis externa needs treatment with antibiotics (e.g.; ceftazidime, mezlocillin, ciprofloxacin), and often surgical treatment[4,5]. However, associated antibiotic resistance makes the choice of treatment difficult. Pseudomonas is known to be highly antibiotic resistant and able to grow through its ability to form resilient biofilms and bacterial cells through the generation of reactive oxygenintermediates[6,7].

Ciprofloxacin is a broad spectrum antibiotic against both gram positive and gram negative bacteria. It inhibits DNA gyrase and topoisomerase, thereby inhibition of cell division. Monotherapy using ciprofloxacin for 6 weeks has been reported effective[4]

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Gentamicin is an aminoglycoside and this antibiotic is active against both gram positive and gram negative bacterial. It is a bactericidal that works by irreversibly binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis. Important factors such as dose, frequency, levels and duration of therapy can affect level of toxicity[9].

Ampicillin is a beta-lactam antibiotic that is part of the aminopenicillin family, active against many Gram-(+) and Gram-(-) bacteria [5]. It is effective for ear infections and respiratory infections but resistance to ampicillin is increasingly common among the bacteria responsible for these infections[9,10].

Otitis media is common in our society and may be a factor of hearing loss. Due to high prevalence of *pseudomonas* in our environment, subjects may have a risk of developing infections including ear, skin etc. So there is a need to find out the antibiotic which is most effective against the middle ear infection and may prevent the patient form hearing loss.

Study was designed to identify and antibiotic susceptibility of pseudomonas isolates from samples taken from the ear.

MATERIALS AND METHODS

This study was conducted on samples received from out door departments of Sir Ganga Ram Hospital, Lahore for a period of 6 months (from Jan 2014 to June 2014). Total 30 ear samples were tested to isolate *Ps. Aeruginosa.* All the samples were transferred to the laboratory soon after collection. The smears were prepared from all the samples on clean glass slides. For identification of *Ps. aeruginosa* biochemical tests like oxidase test and catalase test, sugar (lactose) fermentation test etc., were done. All the samples were inoculated on MacConkey's agar and tested for antibiotic sensitivity.

Isolates of *pseudomonas* areuginosa were tested for antipseudomonal antibiotics including Gentamycin (10µg), Ciprofloxacin (10µg) and ampicillin (10µg) purchased locally. The Kirby-Bauer modified disc diffusion technique was used to determine the sensitivity of the antibiotics. The plates were then incubated at 37°C for 24 hours. The inhibition zone diameter (IZD) were subsequently measured using meter rule and results were recorded as "sensitive" and "resistant". Results were presented in frequency and percentages.

RESULTS

In 30 samples of ear swab the percentage of *pseudomonas* (Ps. Aeruginosa) was 75%. Ps. Aeruginosa gave positive results with oxidase test and negative results with catalase test and sugar lactose fermentation test.

Sensitivity and resistance of antibiotics against *pseudomonas* isolated from ear swab is tabulated (Table). It was observed that sensitivity of ciprofloxacin was 70% and resistance was 29.41% against *pseudomoanas*. Sensitivity of gentamicin was 17.64% and resistance was 82.35% against *pseudomoanas.*On the other hand sensitivity of ampiclillin was11.76% and resistance was 88.23% against *pseudomoanas*.

Table: Sensitivity and resistance of antibiotics against *Pseudomonas* isolated from ear swab

Antibiotics	**No. of sensitivity**	**%age**	**No. of resistant**	**%age**
Ciprofloxcin CIP)	12	70.58	5	29.41
Gentamicin (CN)	3	17.64	14	82.35
Ampicillin-Clavulanic acid (AMC)	2	11.76	15	88.23

DISCUSSION

Present study used MacConkey's agar medium for the growth of *Pseudomonas* and founded that *Pseudomonas* were present with a percentage 70.58 that showed contradiction from a study by 18.82% of pseudomonas used both MacConkey's agar and Blood agar plates. The reason for the contradiction was environmental factor, pollution and nosocomial infection etc[10].

According to our study the highest resistance for ampicillin (88.2%), and highest sensitivity was seen in ciprofloxacin (70.58%). A study also reported that ciprofloxacin shows the highest zone of inhibition against pesuodomonase where as augmentin and gentamycin showes highest resistance against pseudomonas with the least zones of inhibition[11]. On the other hand a group of workers observed that P*seudomonas* was resistant to Ampicillin, Amoxicillin and Tetracycline and highly sensitive to Chloramphenicol and Ciprofloxacin[12]. However it is reported that most strains were susceptible to ciprofloxacin (88.5%) and cefepime (60.6%). The highest resistance was observed to enrofloxacin (51.9%) and gentamicin (43.3%)[8,13]

It is proposed that infection due to P*seudomonas* is able to take hold due to loss of the integrity of a physical barrier to infection (e.g. skin, mucous membrane) or the due to lack of immunity. This bacterium has required minimal nutritional requirements and can tolerate a wide variety of physical conditions like temperatures up to 41^0c[14].

Another study reported that *Pseudomonas* aeruginosa is a leading cause of disorder of the middle ear (chronic otitis media) characterized by infection and discharge. The survivors may suffer from hearing loss and neurological sequelae[15]. Its mode of action is based on three stages i.e. bacterial attachment and colonization, local infection, and bloodstream dissemination and systemic disease[16].

Like other study, we also noted that pseudomonas showed 29.41% resistance against *ciprofloxacin.* A study also reported the resistant strains of *Pseudomonas* organisms against ciprofloxacin (12%) which is of much concern. However it is reported that most strains were susceptible to ticarcillin/clavulanic acid (89.4%), followed by ciprofloxacin (88.5%) and cefepime (60.6%). The highest resistance was observed to enrofloxacin (51.9%) and gentamicin (43.3 %)[13].

CONCLUSION

Ciprofloxacin has been stated to be the most effective drug for the treatment of P. aeruginosa infections. Furthermore, the high rate of resistance to gentamicin and ampicillin recorded in this study appears to confirm the previous suggestions that these two drugs should no longer be taken for the treatment of otitis externa.

REFERENCES

1. Yamamoto N, Nakagawa T and Ito J. Application of insulin-like growth factor-1 in the treatment of inner ear disorders. *Front Pharmacol.* 2014; 5: 208.
2. Schaeefer P and Baugh RF. Lister, P.D. Acute Otitis Externa: An Update. Am Fam Physician. 2012;86(11):1105-61
3. Hajjartabar M. Pseudomonas aeruginosa isolated from otitis externa associated with recreational waters in some public swimming pools in Tehran. Iranian *Journal of Clinical Infectious Diseases* 2010;5(3):142-151.
4. Bugden DL. Identification and antibiotic susceptibility of bacterial isolates from dogs with otitis externa in Australia. *Aust Vet J.* 2013;91(1-2):43-6
5. Paul M1, Lador A, Grozinsky-Glasberg S, Leibovici L. Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis. Cochrane Database Syst Rev. 2014 Jan 7;1:CD003344.
6. Lewis K. Programmed Death in Bacteria. *Microbiol Mol Biol Rev*. Sep 2000; 64(3): 503–514.
7. Barakate A, Kertesz M, James J, Bennett M. "Malignant otitis externa: experience with hyperbaric oxygen therapy". *Diving and Hyperbaric Medicine : the Journal of the South Pacific Underwater Medicine Society*. 2010;40 (4): 195–200
8. Moulds, Robert and Jeyasingham, Melanie. "Gentamicin: a great way to start". *Australian Prescriber*. 2010*;* (33): 134–135.
9. Vydrin, AF. "Component Composition of Gentamicin Sulfate Preparations." *Pharmaceutical Chemistry Journal.* 2003 37: 448-49.
10. Parmar H, Dholakia A, Vasavada D and Singhala H. The current status of antibiotic sensitivity of pseudomonas aerucinosa isolated from various samples.*Int J Res Med.* 2013; 2(1);1-6
11. Venkata Nagaraju E and Divakar G. Antibiotic Susceptibility of Bacterial Strains
12. Isolated from Diabetic Patients. *IJAPBC.* 2012; 1(4):546-50
13. Abbas HA, EL- Masry EM, Shaker GH and Mohsen I. Bacterial etiology and antimicrobial resistance of burn wound infections in a Burn unity in Hehia General Hospital in Egypt. *International Journal of Biological & Pharmaceutical Research.* 2013; 4(12): 1251-1255.
14. Mekić S, Matanović K, Šeol B. Antimicrobial susceptibility of Pseudomonas aeruginosa isolates from dogs with otitis externa. *Vet Rec*. 2011 Jul 30;169(5):125.
15. Mittal R, Grati M, Gerring R, Blackwelder P, Yan D, Li JD, Liu XZ. In vitro interaction of Pseudomonas aeruginosa with human middle ear epithelial cells. *PLoS One*. 2014; 9(3):e91885.
16. Pollack M. *Pseudomonas aeruginosa*. In: GL Mandell,JE Benett, R Dolin (editors). Principles and practice of infectious diseases. New York: Churchill Livingstone. 1995 p. 1980-2003
17. Mozafari Nia, Sepehri G, Khatmi H, Shakibaie MR. Isolation and antimicrobial susceptibility of bacteria from chronic suppurative otitis media patients in kerman, iran. *Iran Red Crescent Med J.* 2011 Dec;13(12):891-4.