



Volume: 3, Issue: 7, 31-34
July 2015
www.biosciencejournals.com
ISSN: 2321-9122
Impact Factor: 3.742

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Susceptibility pattern of *Pseudomonas aeruginosa* to aminoglycosides (Gentamicin and Amikacin) in a tertiary care hospital of Karachi, Pakistan

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Abstract

Objective: To deduce the resistance pattern of multidrug resistant *P.aeruginosa* isolates to aminoglycosides (Amikacin and Gentamicin) from clinical specimens obtained from a tertiary care hospital in Karachi, Pakistan

Methods: A prospective cross-sectional study was undertaken from January 2014 to January 2015 at a private tertiary care hospital in Karachi. Collection of specimens was carried out from three different units across the city. Customary and precise bacteriological procedures were used to detect the clinical isolates. The isolates were cultured on chocolate and MacConkey agar. Utilizing the Kirby Bauer Disc diffusion method on Mueller-Hinton Agar the sensitivity patterns were deduced

Results: A total of 1622 isolates of *P. aeruginosa* were cultured during the study's time frame spanning over a year. Cultures which were found to be positive were then tested against Amikacin and Gentamicin and their pattern of susceptibility were ascertained.

Conclusions: MDR *P. aeruginosa* isolates show an advanced pattern of resistance as compared to previous studies. The amplified pattern of resistance of isolates to Amikacin and Gentamicin was observed.

Keywords: Susceptibility pattern, *Pseudomonas aeruginosa*, Gentamicin and Amikacin, tertiary care hospital, Karachi Pakistan

Introduction

Pseudomonas aeruginosa is a cosmopolitan gram-negative aerobic bacillus which is oxidase positive ^[1] isolated from soil, water, plants, and animals including humans. *P. aeruginosa* is physiologically flexible and grows as a saprophyte in various environments including basins, drainage system and antiseptic solutions ^[2]. It is an opportunistic pathogen which causes nosocomial infections primarily in immunocompromised patients. A disruption in the integrity of physical barriers to bacterial invasion, such as intravenous lines, urinary catheters, or endotracheal tubes may contribute to its spread. *P. aeruginosa* can cause infections virtually anywhere in the body but UTI, pneumonia (esp ventilator associated and cystic fibrosis patients) and wound infection (burns) predominate. It is fundamentally invulnerable to various antimicrobial agents owing to membrane permeability, multi-drug efflux, acquisition of plasmids, transposons or integrons encoding aminoglycoside-modifying enzymes ^[3].

MDRP strains are namely those that have developed resistance against aminoglycosides, carbapenems and fluoroquinolones ^[4]. Multiple factors contribute to *P. Aeruginosa* attaining rapid resistance to all antibiotics used against them, amongst them formation of biofilms, with oxygen limitation and low metabolism in the interior rather than poor antibiotic penetration ^[5]. Another factor being, an active efflux system, the MexXY helps *P. Aeruginosa* to attain intrinsic resistance to Aminoglycosides ^[6]. Aminoglycosides antibiotics compromise translation by accumulation of aberrant polypeptides. The latter jeopardize cytoplasmic membrane integrity and causes accumulation of drugs and inhibits all cellular ribosomes, further damage is accentuated by production of ROS. All these factors culminate to have a lethal effect on bacterial cells ^[7]. *P. Aeruginosa* was found to develop eventual resistance to all antibiotics used against them with the exception of amikacin and piperacillin/tazobactam ^[8]. Other successful combinations include ciprofloxacin with amikacin and gentamicin which has a synergistic effect against MDR *P. Aeruginosa* and no antagonistic effects ^[9].

Materials and Methods

This study was conducted over a time period of 1 year in a tertiary care hospital of Pakistan in the microbiology department of Ziauddin Medical University from January 2014 to January 2015. Inpatients who were admitted in the ICU and HDU had their body fluids, urine, bronchoalveolar lavage, Tracheal aspirate, blood and pus samples collected. Selected patients comprised of all age groups. The samples were incubated for duration of 18 hours at 37 °C on Chocolate and MacConkey agar. Standard microbiological methods were used to identify isolates of *P. aeruginosa* by its colony morphology. On gram staining it is a pleomorphic gram negative rod, non-lactose fermenter on MacConkey, pigment production with characteristics grape like odor, oxidase positivity, non-motile along with its ability to reduce nitrate to nitrite and arginine decarboxylase and gelatin liquefaction. Confirmation of isolates was done by using Api20 NE. using Kirby Bauer Disc Diffusion method on Mueller – Hinton agar, by following CLSI 2014 Guidelines and using Hi-media antibiotic discs antibiotic sensitivity and resistance pattern were determined. Antibiotics were tested, which included Amikacin (30mcg), Gentamicin (30mcg), Ceftazidime (30mcg), Aztreonam (30mcg), Cefepime (30mcg), Cefepime/sulbactam (75/30mcg), Ciprofloxacin (5mcg), Piperacillin + Tazobactam (100/10mcg), Meropenem (10mcg) and Colistin (10mcg). For quality control in the study ATCC 27853 strain was used. Statistical analysis was performed by IBM SPSS version 22. Frequency and percentage of resistant antibiotics were calculated. *P. aeruginosa* was detected as a bacterium which was resistant to three or more anti-Pseudomonal antimicrobial classes (Piperacillin+ Tazobactam, Imipenem, Ceftazidime and Amikacin) was determined as MDR in our work.

Results and Discussion

Pseudomonas aeruginosa belongs to a class of bacteria known as Gamma Proteobacteria. *Pseudomonas aeruginosa* is the epitome of an opportunistic pathogen of humans. The characteristic *Pseudomonas* bacterium in its natural habitat might be found in a biofilm, a planktonic form, attached to some surface or substrate, as a unicellular being, swimming actively by the help of its flagellum. *P. aeruginosa* is well known for its metabolic flexibility which it possesses. Nosocomial infections represent major sources of morbidity and mortality for patients in ICU [10, 11] Important risk factors for such infections include life threatening medical or surgical conditions, the immunocompromised state, alterations in flora due to exposure to multiple antibiotics, and the disruption of skin and mucous membranes by use of invasive devices [12, 13].

Aminoglycosides are a vital component of antipseudomonal chemotherapy implicated in the treatment of a variety of infections [14]. maximum sensitivity was noted against *Pseudomonas* species when the aminoglycoside group of antibiotics-amikacin was used against it. Amikacin was designed as a poor substrate for the enzymes that bring about inactivation by phosphorylation, adenylation or acetylation. Amikacin seems to be a promising therapy for Pseudomonal infection. Hence, its use should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains [15].

Numerous studies conducted in the past suggest an alarmingly increasing resistance pattern to the various

antibiotics used in the treatment of the infections caused by *Pseudomonas aeruginosa*. The decreased susceptibility of the notorious bacteria to virtually all antibiotics used against it is another major contributing problem. Our research focuses on two antibiotics belonging to the aminoglycoside family; Gentamicin and Amikacin.

The data suggests that out of 1662 isolates, 30.5%(507) of the isolates were resistant to gentamicin whereas 69.5%(1155) of the isolates were sensitive to it as shown in Figure 1. and Table 1. While Table 2 and Figure 2. depicts that 26.5%(440) isolates were resistant to amikacin and 73.5% (1222) were sensitive.

Although Notably high resistance, 67.86% was noted towards gentamicin, and 50% to amikacin by *Pseudomonas aeruginosa* in a study conducted by Javiya in 2008 in India [16]

Our results correspond to the study carried out by Walky *et al.* 2011, Antimicrobial susceptibility of *pseudomonas aeruginosa* isolates obtained from patients in Canadian hospitals; CANWARD 2008-2011, where the resistance to gentamicin was calculated to be 23.2%, and that to amikacin was 8% [17].

The data obtained from the National Surveillance of Antimicrobial Resistance in *pseudomonas Aeruginosa* isolates obtained from ICU patients from 1993-2002 documents that in the year 2000, *Pseudomonas aeruginosa* accounted for 32% resistance to Gentamicin after which the drug was discontinued for the next two years due to increasing resistance. Amikacin resistance were 9% in 2000, 13% in 2001 and 10% in 2002 [18].

Our study corroborates with a similar report published by Vojtova *et al.* 2011 which shows resistance to gentamicin as 33.1% and that to amikacin as 4.8% [18].

Another recent research conducted in 2013 by Begum *et al.* in Bangladesh depicts gentamicin resistance to be at 40% and amikacin at 36.3% which is notably high resistance pattern observed in the south Asian region [19].

This extremely high resistance observed is most likely due to the non-judicial use of antibiotic, which is considerably frequent in the said regions and the leading cause of emergence of highly resistant strains of *Pseudomonas aeruginosa* which are posing a serious problem for not only healthcare professionals but pharmaceutical companies as no recent antimicrobials are under investigation other than those currently available.

Figures and Tables

Resistance and Sensitivity pattern of *P.aeruginosa* to Gentamicin(n=1662)

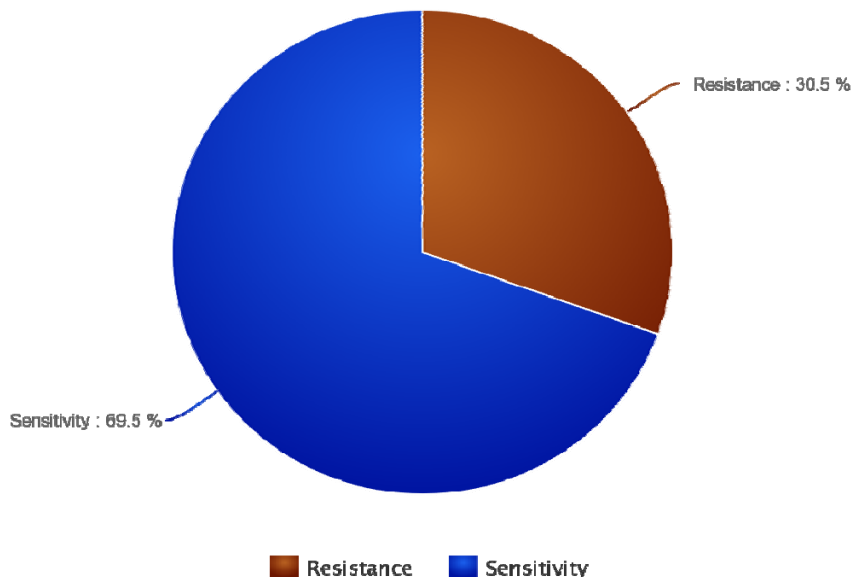


Fig 1

Resistance and Sensitivity pattern of *P.aeruginosa* to Amikacin(n=1662)

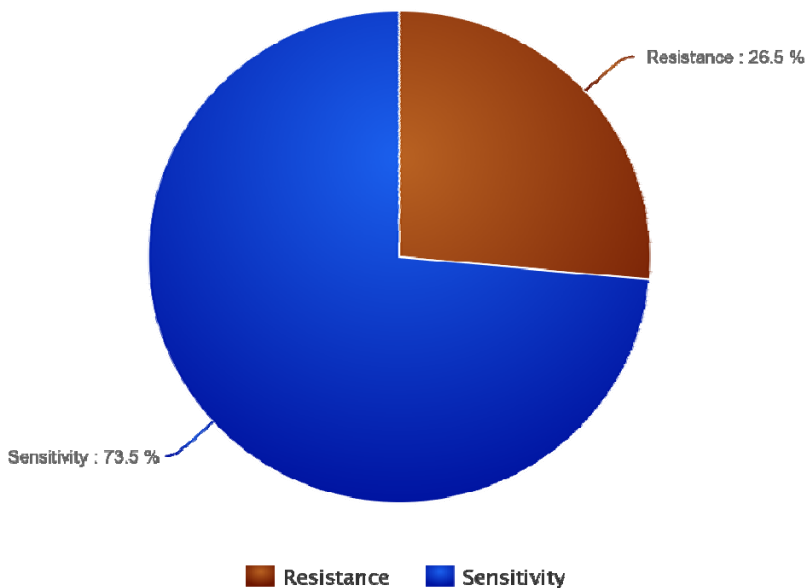


Fig 2

Table 1: Sensitivity and resistance pattern of isolates to Gentamicin

Pattern	Frequency	Percent	Valid Percent
Resistant	507	30.5%	30.5%
Sensitive	1155	69.5%	69.5%
Total	1662	100%	100%

Table 2: Sensitivity and resistance pattern of isolates to Amikacin

Pattern	Frequency	Percent	Valid Percent
Resistant	440	26.5%	26.5%
Sensitive	1222	73.5%	73.5%
Total	1662	100%	100%

Conclusion

It has been observed that irrational and inappropriate prescription and use of antibiotics is rampant in the south Asian countries and has contributed majorly to rapid emergence of resistant strains to presently available drugs used for their treatment.

Another contributing factor is the use of reserved drugs such as amikacin for treatment of mild infections that may or may not be caused by the *Pseudomonas aeruginosa*.

All these factors demand immediate actions to curtail this grievous problem. Misuse of antibiotics must be halted in order to preserve the efficacy and potency. This can be

undertaken by setting up a system comprised of state endorsed drug usage policy accompanied by guidelines, effective immediately. And thus maintain adequate patient management.

Further improvement can be ensured by setting up a surveillance system to record emerging resistance among *Pseudomonas aeruginosa* to available drugs.

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