Original article

Detection of aminoglycosides resistance among strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from patients with urinary tract infection

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Abstract

This study aims to detect the resistance pattern of aminoglycosides among isolated strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* from patients with urinary tract infection (UTI). A total of 70 urine samples were collected from patients with symptoms of urinary tract infection in sterile urine containers, and then inoculated in CLED media, incubated at 37 °C for 24 hours. Species of *K. pneumoniae* and *P. aeruginosa* were identified according to their colonial morphology, indirect gram staining reaction and biochemical tests. Identified species were tested for antimicrobial susceptibility against the following aminoglycosides: Amikacin, Kanamycin, Streptomycin, Gentamicin, and Tobramycin using the Kirby-bauer disc diffusion method. Results revealed that the overall resistance of *K pneumoniae* to aminoglycosides was 16% and 33.3% for *P. aeruginosa*. In conclusion, the most powerful aminoglycoside against *K. pneumoniae* isolated from UTI patients was Gentamicin, Amikacin and Kanamycin, and the most powerful antibiotic against *Pseudomonas* species was Amikacin and Gentamicin respectively.

Keywords: Aminoglycosides, resistance, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, UTI.

Introduction

Urinary Tract Infections (UTI) are widespread in communities and hospitals in the world. There are two types of UTI, symptomatic and asymptomatic. Symptomatic infection is associated with a wide range of morbidity from mild irrigative voiding symptoms to bacteremia, sepsis and occasionally death. Asymptomatic UTI is the isolation of bacteria from urine in quantitative counts consists with infection but without localizing genitourinary signs and symptoms [1].

The most common cause of UTI is the species of *Enterobacteriacae* family, causing 84.3% of the UTIs. *Escherichia coli* causes about 85% of community-acquired UTIs, 50% of nosocomial UTIs and more than 80% of cases of uncomplicated pyelonephritis [2].

Multiple antimicrobial resistance among gram-negative organisms has been a long term and well-recognized problem with urinary tract infections. Resistance has been observed in multiple genera including *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia* and *Pseudomonas* [2]. The mechanisms whereby bacteria circumvent drug action are many and varied, ranging from intrinsic impermeability to acquired resistance involving plasmids transposons, and mutations [2]. *Klebsiella* spp. are gram-negative, non-motile, usually encapsulated

rod-shaped bacteria, belonging to the family Enterobacteriaceae; members of this family are generally facultative anaerobic [3]. *P. aeruginosa* is a gram-negative rod measuring 0.5 to 0.8 micrometer by 1.5 to 3.0 micrometer. Almost all strains are motile by means of a single polar flagellum [4].

Urinary tract infections are one of the major causes of morbidity and co-morbidities in patients with underlying conditions, and it accounts for the majority of the reasons for hospital visits globally. Sound knowledge of factors associated with UTI may allow intervention that can easily bring the disease under control [5]. Therefore, the current study is conducted to detect the percentage of resistance of *K. pneumoniae* and *P. aeruginosa* strains that cause UTI to selected aminoglycosides.

Materials and methods

Sampling

A total of 70 urine samples were collected consecutively from in and out-patients at Shendi Teaching Hospital and Almak Nimer University Hospital, Shendi, Sudan. The collection was done by trained medical personnel avoiding contamination. Clean-catch mid-stream urine samples were collected from consenting patients. The samples were immediately transported to the laboratory after collection and processed. All contaminated urine specimens and all patients who refused to fill the consent form were excluded from the study [6,7].

Isolation and identification

A loop-full (0.001 ml) of well-mixed uncentrifuged urine was streaked onto the surface CLED agar. The plates were incubated aerobically at 370 °C for 18-24 hours and counts were expressed in colony forming units (CFU) per milliliter (mL). A count of 10^5 CFU/Ml or more was considered significant bacteriuria. Strains of *K. pneumoniae* and *P. aeruginosa* was identified by colonial morphology, indirect gram stain and biochemical tests [8].

Antimicrobial susceptibility testing

This was done using the Kirby Bauer Disc diffusion method concerning the Clinical Laboratory Standard Institute (CLSI) performance guideline for antimicrobial susceptibility testing. Quality was assured by testing the *E. coli* quality control strain, ATCC 25922, in every batch. All zones of inhibition determined were within the ranges prescribed by the CLSI. Five aminogycosides were applied; Tobramycin 10mg, Kanamycin 30mg, Streptomycin 10 mg, Gentamicin 10mg, and Amikacin 30mg [9].

Results

A total of 70 participants were included in this study; the majority of them, 84.3%, were females and 15.7% were males**.** Their age ranged from 4 to 67 years, more than 41%

were within the age group 31-60 years. While the age group 1-30 years represent 25.7% and above 60 years was 32.9%. The *K. pneumoniae* was found responsible for 7% of the UTI, likewise, the same figure for *P. aeruginosa*. While other pathogens represent 86%.

The resistance of isolated *K. pneumoniae* to aminoglycosides was 60% to Tobramycin, 0% to Kanamycin, 20% to Streptomycin, 0% to Gentamycin, and 0% to Amikacin. The isolated species of *Klebsiella* show the highest resistance to Tobramycin and the lowest resistance level was detected against Gentamycin, Kanamycin and Amikacin. The overall resistance of isolated *Klebsiella* species was 16 %.

The resistance of isolated *P. aeruginosa*to to aminoglycosides was 25% to Tobramycin, 60% to Kanamycin, 40% to Streptomycin, 20% to Gentamycin and 20% to Amikacin. The isolated species of *Pseudomonas* show highest resistance to Kanamycin and the lowest resistance level was detected against Gentamycin and Amikacin. The overall resistance of isolated *Pseudomonas* species was 33.3%.

Discussion

This study was conducted to detect aminoglycosides resistance among *Klebsiella* and *pseudomonas* species isolated from patients with urinary tract infection. The study shows that the infection was highest in females compared to males. This is similar to a previous study that showed that UTI more commonly occurred in females than males [9]. The prevalence of bacterial UTI was highest in the age group 31-60 years. This finding disagrees with the result obtained in Uganda that the prevalence was highest in the age group 20-29 years. This difference may be due to that most samples collected were from pregnant UTI patients who are mainly found in this age group [5].

The study denoted that *Klebsiella* species was responsible for 7% of UTI. This is in agreement with a result that the *Klebsiella* species was responsible for 9.7% of UTI [10]. Likewise, the *Pseudomonas* species was responsible for 7% of UTI. This result is near to that previously obtained that the prevalence of *pseudomonas* species was 5.4% [11].

In this study, the resistance of *Klebsiella* species to Tobramycin was 60%. This finding is near to a previous result that the resistance of this species to Tobramycin was 53.1% [10].

This study found that there is no reported resistance for *Klesiella* species to Gentamicin, Amikacin and Kanamycin. This result was in difference with results obtained by other researchers who reported that the percentage of resistant among *Klesiella* species to Gentamicin was 33.3%, to Amikacin was 36.96% and to Kanamycin

was 16% [2,12,13]. This difference may be justified by the difference in the antibiotic prescription policy applied in different areas, in our country the prescription policy relying on cephalosporins rather than aminoglycosides which may denote the less resistance in aminoglycosides rather than other classes of antibiotics. The resistance of *Klebsiella* species to Streptomycin was 20%. This finding is in different with result previously obtained that the percentage of resistance among *Klesiella* species to Streptomycin was 87.6% [14].

In this study, the resistance of *Pseudomonas* species to Tobramycin was 25%. This finding is different from the result that the resistance of *Pseudomonas* species to Tobramycin was 54.3% [15]. The resistance of *Pseudomonas* species to Gentamicin was 20%. This result was in difference with a result that the percentage of resistance among *Pseudomonas* species to Gentamycin was 13.88% [9]. The resistance of *Pseudomonas* species to Streptomycin was 40%. This finding is in different with a result that the percentage resistance among *Pseudomonas* species to streptomycin was 47% [2]. The high resistance rate in this study may refer to the lake of health system for usage of antibiotic. The resistance of *Pseudomonas* species to Amikacin was 20%. This finding is in agreement with a result that the percentage resistance among *Pseudomonas* species to Amikacin was 25.3% [11]. The resistance of *Pseudomonas* species to Kanamycin was 60%. This finding is in different with a previous result that the percentage resistant among *Pseudomonas* species to Kanamycin was 17.3% [16].

In conclusion, the study revealed that the overall resistance of *K. pneumoniae* to aminoglycosides was 16% and the overall resistance of *P. aeruginosa* to aminoglycosides was 33.3%.

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