ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3
AJCEM/1822 http://www.ajol.info/journals/ajcem
COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.1
AFR. J. CLN. EXPER. MICROBIOL. 19 (3): 159-164

# ANTIMICROBIAL RESISTANCE PATTERN OF CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* AND *ESCHERICHIA COLI* ON CARBAPENEMS

**Oladipo E.K.[1, 2], Ajibade O.A. [1, 2*], Adeosun I.J[1], Awoyelu E. H.[2], Akinade S.B. [2], Alabi O.A.[2] and Ayilara O. A.[2,3]**

**[1]Department of Microbiology, Adeleke University, P.M.B 250, Ede, Osun State, Nigeria; [2]Department of Pure and Applied Biology (Microbiology/Virology Unit), Ladoke Akintola University, P.M.B 4000, Ogbomoso, Nigeria; [3]Department of Medical Microbiology and Parasitology, Ladoke Akintola University Teaching Hospital , Oshogbo, , Osun State, Nigeria**

**Correspondence: O.A Ajibade. Adeleke University, P.M.B 250 Ede, Osun State E – Mail: oluwatosin.ajibade@adelekeuniversity.edu.ng**

## ABSTRACT

**Background:** Carbapenems are the most effective and important therapeutic options to serious infections caused by *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates. However, Carbepenems resistant isolates of *Enterobacteriaceae* and *Pseudomonas aeroginosa* are increasing worldwide. This study, therefore, was carried out to determine the resistance pattern of clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* to Carbapenems.

**Methods:** Fifty (50) *E. coli* and forty seven (47) *Pseudomonas aeruginosa* isolates were studied. Antibiotic Susceptibility test was performed as recommended by the CLSI. The antibiotics used were Ertapenem, Imipenem, Colistin Sulphate, Levofloxacin, and Piperacillin/Tazobactam.

**Results:** Out of 97 clinical isolates subjected to drug susceptibilities test, *Pseudomonas aeruginosa* showed resistance to Ertapenem (87.2%); followed by Levofloxacin (19.1%), Colistin sulphate (12.8%), Piperacillin/tazobactan (4.3%) and Imipenem (2.1%) while *E.coli* displayed resistance to Ertapenem (30%), Levofloxacin (20%) and Colistin sulphate (4%). Interestingly, *E coli* was susceptible to Imipenem (0%) and Piperacillin/tazobactan (0%). A significant effect of Ertapenem on *Pseudomonas aeruginosa* was recorded. Also a significant effect of Piperacillin/Tazobactam was recorded on *E coli*. No significant effect was recorded among the other antibiotics on *P aeruginosa* or *E coli*.

**Conclusion:** There is a high level of Carbapenems resistance among the clinical isolates of *Pseudomonas aeruginosa* compared to *Escherichia coli* in this study. Considering the therapeutic value of Carbapenems as one of the last options for the treatment of *Enterobacteriaceae* and *Pseudomonas aeruginosa* infections, rational Carbapenems usage is essential to reduce selective pressure over *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates.

**Keywords: Carbapenems, Antibiotics, Nosocomial, Susceptibility**

# PROFIL DE RÉSISTANCE AUX ANTIMICROBIENS DES ISOLATS CLINIQUES DE PSEUDOMONAS AERUGINOSA ET ESCHERICHIA COLI SUR CARBAPÉNÈMES

**Oladipo E.K.[1, 2], Ajibade O.A. [1, 2*], Adeosun I.O[1], Awoyelu E. H.[2], Akinade S.B.[2], Alabi O.A.[2]**

**and Ayilara O. A.[2]**

**1Département de microbiologie, Université Adeleke, P.M.B 250, Ede, Osun State, Nigeria ; 2Service de biologie fondamentale et appliquée (Microbiologie/virologie Unité), l'Université Ladoke Akintola, P.M.B Ogbomoso, 4000, au Nigeria. [3]Department of Medical Microbiology and Parasitology, Ladoke Akintola University Teaching Hospital , Oshogbo, , Osun State, Nigeria**

**Correspondance: O.A Ajibade. Adeleke University, P.M.B 250 Ede, Osun State E - Mail: oluwatosin.ajibade@adelekeuniversity.edu.ng**

## RÉSUMÉ

**Contexte : Carbapénèmes sont les plus efficaces et les options thérapeutiques importants d'infections graves causées par les entérobactéries et Pseudomonas aeruginosa isolats. Cependant, Carbepenems isolats résistants d'entérobactéries et Pseudomonas aeroginosa sont en augmentation dans le monde entier. En conclusion, cette étude a été réalisée pour déterminer le profil de résistance des isolats cliniques de Pseudomonas aeruginosa et Escherichia coli de carbapénèmes.**

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**Méthodes: Cinquante (50) E. coli et quarante sept (47) des isolats de Pseudomonas aeruginosa ont été étudiés. Test de sensibilité aux antibiotiques a été effectuée comme recommandé par le QIMV. Les antibiotiques utilisés étaient l'ertapénème, imipenem, sulfate de colistine, Lévofloxacine, et de l'association pipéracilline-tazobactam.**
**Résultats : Sur 97 isolats cliniques de médicaments soumis à des test de susceptibilité, Pseudomonas aeruginosa ont montré une résistance à l'ertapénème (87,2 %), suivie par la lévofloxacine (19,1 %), sulfate de colistine (12,8 %), l'association pipéracilline-tazobactam (4,3 %) et l'imipénème (2,1 %) tandis que les E. coli affiche une résistance à l'ertapénème (30 %), la lévofloxacine (20 %) et sulfate de colistine (4 %). Fait intéressant, E coli était sensible à l'imipénème (0 %) et de la pipéracilline/tazobactam (0 %). Un effet significatif de l'ertapénème sur Pseudomonas aeruginosa a été enregistrée. Aussi un effet significatif de l'association pipéracilline-tazobactam a été enregistré sur E coli. Aucun effet significatif n'a été enregistré parmi les autres antibiotiques sur *P aeruginosa* ou *E coli.***

**Conclusion : Il y a un haut niveau de résistance aux carbapénèmes parmi les isolats cliniques de Pseudomonas aeruginosa par rapport à Escherichia coli dans cette étude. Compte tenu de la valeur thérapeutique des carbapénèmes comme l'une des dernières options pour le traitement d'entérobactéries et Pseudomonas aeruginosa les infections, l'utilisation rationnelle des carbapénèmes est essentielle pour réduire la pression sélective sur les entérobactéries et Pseudomonas aeruginosa isolats cliniques.**

**Mots-clés : Carbapénèmes, antibiotiques, infections nosocomiales, la sensibilité**

## INTRODUCTION

Antibiotic resistance of pathogenic organisms has become a worldwide problem with serious concern both in hospital and community settings posing threatening consequences on the treatment of infectious diseases. The increased use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon (1, 2). *Pseudomonas aeruginosa* is one of the most frequent (10-20%) pathogens associated to nosocomial infections, especially among immunocompromised patients (2) exhibiting notorious versatility and capacity to acquire resistance mechanisms to antimicrobial therapy(3). Within the hospital, *P. aeruginosa* finds numerous reservoirs in disinfectants, respiratory equipment, food, sinks, taps and mops. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated reservoirs, and by ingestion of contaminated foods and water (4).

Mechanisms of resistance to Carbapenems in *P. aeruginosa* are impermeability, including closure of porin channels in the bacterial cell wall; and extrusion of antibiotics by efflux pumps, which can lead to resistance to multiple classes of antibiotics. *E. coli*, on the other hand, produce extended-spectrum β-lactamases (ESBLs) which may develop on the basis of a change in only one amino acid in the β-lactamases normally produced (5). By contrast to plasmid-mediated production of ESBLs, AmpC β-lactamases are chromosomally-mediated and occur in ICU pathogens such as *P. aeruginosa*, *Enterobacter spp*, and in recent years in *E. coli* (6).

Carbapenems are a class of potent β-lactams considered as the last resort option for treating serious nosocomial infections caused by a broad spectrum of Gram-negative bacteria. They are known not to easily diffuse through the bacterial cell wall (7). They enter the Gram-negative bacteria through the outer membrane proteins (OMPs), after transversing the periplasmic space; Carbapenems 'permanently' acylate the penicillin-binding proteins (PBPs) which are enzymes that catalyze the formation of peptidoglycan in the cell wall of bacteria (8, 9, 10). Carbapenems act as mechanism-based inhibitors of the peptidase domain of PBPs which inhibits peptide cross-linking as well as other peptidase reactions. Carbapenems are prominent for their ability to bind to multiple different PBPs and eventually weakening the peptidoglycan ultimately leading to cell burst due to osmotic pressure (8, 11).
Carbapenems resistance is modulated by acquired carbapenemases in association with intrinsic mechanisms such as down-regulation or loss of OPrD porin, efflux pumps hyperextension, chromosomal AmpC β-lactamase production, and target alterations. However, since carbapenemases have the ability to hydrolyse Carbapenems, Gram-negative bacteria carrying a carbapenemase-encoding gene frequently exhibit resistance to virtually all β-lactams.

Majority of the non-fermenting Gram-negative bacteria (e.g., *pseudomonas* spp., *Acinetobacter* spp., and *Stenotrophomonas* spp.), as well as the *Enterobacteriaceae* (e.g., *Klebsiella* spp., *Escherichia coli*, and *Enterobacter* spp.) and Gram-positive bacteria (e.g., *Staphylococcus* spp., *Streptococcus* spp., *enterococcus* spp., *Nocardia* spp.), have shown resistance to most clinically available carbapenems and this is of grave public health concern (12).
Given the importance of Carbapenem for the treatment of infections caused by *P. aeruginosa* and *E. coli,* this present study was carried out to examine the antimicrobial resistance pattern of Carbapenems on the clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* in order to determine its efficacy.

## MATERIAL AND METHODS

### Collection of samples

Ninety seven (97) isolates were obtained from the University College Hospital, comprising of fifty (50) *E. coli* and forty seven (47) *Pseudomonas aeruginosa* isolates. They were sculptured onto sterile slant bottles. They were transferred to the Microbiology Research Laboratory of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, South-Western part of Nigeria for further analysis.

### Processing of samples

Inoculum preparation was done under asepsis by picking isolates from the slant bottles into universal bottles containing 5ml of normal saline to obtain a suspension equivalent to the turbidity of 0.5 McFarland standards.

### Antibiotic susceptibility testing

Susceptibility test was determined using the disc diffusion method on Mueller-Hinton agar plates and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014) as described by (13). Five antibiotics were tested, including Ertapenem (10µg), Imipenem (10µg), Colistin Sulphate (25µg), Levofloxacin (5µg), and Piperacillin/Tazobactam (110µg).

### Statistical analysis

Statistical analysis was carried out with the Statistical Package for Social Sciences (SPSS) version 20.0; ANOVA was done at $p$-value < 0.05 and at a confidence interval of 95%.

## RESULTS

Table 1 showed the antibiotics susceptibility profiles of the clinical isolates cum variation using disk-diffusion methods. *Pseudomonas aeruginosa* had the highest resistance rates to Ertapenem (87.2%), followed by Levofloxacin (19.1%), Colistin sulphate (12.8%), Piperacillin/Tazobactam (4.3%) with the least resistance observed in Imipenem (2.1%).

**TABLE 1: THE ANTIBIOTICS SUSCEPTIBILITY PROFILES OF *P. AERUGINOSA* AND *E. COLI***

| *Organisms (isolates)* | *Antibiotics* | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Ertapenem (10µg)* | | | *Imipenem (10µg)* | | | *Colisin sulphate (25µg)* | | | *Levofloxacin (5µg)* | | | *Piperacillin/tazobactam (110µg)* | | |
| | *S* | *I* | *R* | *S* | *I* | *R* | *S* | *I* | *R* | *S* | *I* | *R* | *S* | *I* | *R* |
| *P. aeruginosa(%)* | *4.3* | *8.5* | *87.2* | *4.3* | *93.6* | *2.1* | *6.4* | *80.9* | *12.8* | *80.9* | *0* | *19.1* | *89.4* | *6.4* | *4.3* |
| *E. coli* | *56%* | *14%* | *30%* | *100%* | *0%* | *0%* | *90%* | *6%* | *4%* | *80%* | *0%* | *20%* | *96%* | *4%* | *0%* |

**Key: S= Susceptible, I= Intermediate and R= Resistant**

*E. coli* had the highest resistance rates to Ertapenem (30%) followed by Levofloxacin (20%). The least was recorded in Colistin sulphate (4%). No resistance was recorded in Imipenem and Piperacillin/Tazobactam, respectively. *P. aeruginosa* isolates had highest susceptibility to Piperacillin/Tazobactam (89.4%) followed by Levofloxacin (80.9%), Colistin sulphate (6.4%), with the least recorded in Imipenem and Ertapenem (4.3% each), respectively. *E. coli* had the highest susceptibility to Imipenem (100%) followed by Piperacillin/Tazobactam (96%), Colistin sulphate (90%), Levofloxacin (80%). The least was recorded in Ertapenem (56%). These results suggest that Ertapenem is least effective against *E. coli* and *P. aeruginosa* since about 57.7% of these clinical specimen isolates are resistant to it, while Piperacillin/Tazobactam is most effective because about 92.8% of these organisms are susceptible to it.

The result of ANOVA revealed a significant effect of Ertapenem on *Pseudomonas aeruginosa*. Also a significant effect of Piperacillin/Tazobactam was recorded on *E coli*. No significant effect was recorded among the other antibiotics on *P aeruginosa* and *E coli* (Table 2).

**TABLE 2: ANOVA FOR MEAN EFFECT OF ANTIBIOTICS ON ISOLATES**

| Organisms | Antibiotics | | | | |
|---|---|---|---|---|---|
| | Ertapenem | Imipenem | Colisin sulphate | Levoflox | Piperacillin/tazobactam |
| *P. aeruginosa* | 1.27 ± 0.07$^{b}$ | 1.37 ± 0.02$^{a}$ | 1.95 ± 0.03$^{a}$ | 1.48 ± 0.02$^{a}$ | 0.88 ± 0.00$^{a}$ |
| *E. coli* | 1.24 ±0.40$^{a}$ | 1.84 ± 0.04$^{a}$ | 1.32 ± 0.01$^{a}$ | 1.74 ± 0.02$^{a}$ | 2.32 ± 0.05$^{ab}$ |

**Key: Values are mean scores ± standard error. a, b = Mean values followed by the same superscript in the columns are not significantly different by Duncan's Multiple Range test (P ≤ 0.05).**

**DISCUSSION**

Antibiotic resistance determinants have been circulating within the microbial genome for millennia, largely predating the manufacture and use of antibiotics by human beings (14). Antibiotic resistance correlates well with the frequency of drug use and in a country like Nigeria where drugs are easily available over-the-counter, bacterial resistance to antibiotics grows rapidly, putting our health care system in a dilemma. Drug resistance facilitates growth and increases prevalent of persistent pathogens which become difficult to exterminate (15).

*P aeruginosa* is the most common non-fermenting bacterium isolated from clinical samples posing a serious therapeutic threat for the treatment of both community-acquired and nosocomial infections. Identification and selection of appropriate antibiotic to initiate therapy is essential to optimizing clinical outcome. *E. coli*, on the other hand, produce extended-spectrum β-lactamases (ESBLs) which may develop on the basis of a change in only one amino acid in the β-lactamases normally produced (6). By contrast to plasmid-mediated production of ESBLs, AmpC β-lactamases are chromosomally-mediated and occur in ICU pathogens such as *P. aeruginosa*, *Enterobacter spp*, and in recent years in *E. coli* (14).

From this study, *P aeruginosa* had more resistance to the antibiotics used as compared with *E coli*. The study showed majority (87.2%) of *P aeruginosa* isolates were resistant to ertapenem, followed by levofloxacin (19.1%) and colistin sulphate (12.8%). Similar resistant pattern against the isolate was reported in different studies conducted by [16], [17] and [18]. Imipenem and piperacillin/tazobactam were most effective drug observed in this study showing resistant rates of 2.1% and 4.3% respectively. This pattern is in accordance with the reports of (19, 20, 21).

On the other hand, *E coli* showed a major (30%) resistance to ertapenem followed by levofloxacin (20%) and Colistin sulphate (4%). This is in accordance with the findings of (22, 23, 24). Interestingly, the isolates were 100% susceptible to imipenem and piperacillin/Tazobactam, respectively. This is in accordance and in disagreement to the report of [26] who recorded 100% sensitivity for imipenem and nearly 40% sensitivity to piperacillin/Tazobactam.

In Nigeria, the sensitivity of the isolates to imipenem and piperacillin/tazobactam is in accordance with the reports of (27, 28, 29). The high susceptibility pattern of these drugs could be associated to less drug abuse by the population due to their cost preventing patient's self-medication. However, the pattern disagrees with the findings of [30], [31] where a high resistant was recorded, thus demonstrating the evolution of imipenem-resistant strains of *P. aeruginosa* to imipenem.

One of the reasons for resistance might be due to misuse and overuse of antibiotics, that is, not adhering to the prescription of antibiotics. Also, it can be transferred horizontally between bacteria. *P. aeruginosa* resistance to Carbapenems may be due to a result of complex interactions of several mechanisms including production of carbapenemase, overproduction of efflux system and loss of outer membrane porins (21). *P aeruginosa* resistance to imipenem and piperacillin/Tazobactam might be as a result of movement of various types of patients being referred to UCH, a tertiary hospital, both locally and internationally for management or continuation of therapy, thus the selective pressure of use, misuse and overuse of antibiotics cannot be farfetched. Also, there is the likelihood of transfer of resistance genes from other clinics around the world.

Resistance in *E coli* might be as a result of transfer of plasmids between commensal organisms and potential pathogens through inappropriate or overprescribing of antibiotics and difficulty in establishing bacterial etiology at the time of prescription. Therefore, there is need for public awareness on the prudent use of antibiotics and strict adherence to minimize the misuse of effective drugs. Overall, the data obtained indicate that imipenem and piperacillin/Tazobactam are the most effective for the treatment of *P. aeruginosa* and *E. coli* infections. Though effective, proper monitoring of resistance to these antibiotics is necessary.

**Conclusion:** This study revealed that most of the isolates were susceptible to imipenem and piperacillin/tazobactam. To avoid resistance, illicit use of antibiotics is advised. Continued monitoring of antimicrobial resistance patterns in hospitals and community settings is essential to guide effective empirical therapy. Furthermore, piperacillin/tazobactam may be considered as reserve drug for treatment of *P. aeruginosa* infections.

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