https://dx.doi.org/10.4314/dujopas.v8i4b.3
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 8 No. 4b December 2022*

Assessment of Capsule Related Antibiotic Resistance of *Pseudomonas aeruginosa* Isolated from Patients Attending some Hospitals in Kano State, Nigeria

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Abstract
Resistance to antimicrobial substances by pathogenic microorganisms is on the rise since the beginning of modern medicine. Mechanisms; enzymatic degradation, loss of target and antimicrobial interception are employed by bacteria in resisting antimicrobials. Bacterial capsule is a gelatinous outer layer surrounding a bacterial cell composed primarily of polysaccharide. This study assessed the role that the capsule of Pseudomonas aeruginosa played in carbapenem resistance. P. aeruginosa was isolated from 49 (16%) samples out of 300 samples screened. India ink staining technique was used to detect the capsule. Capsule was detected in 42 (86%) of the isolates whereas 7 (14%) of the isolates were non-capsulated. All the capsulated strains were resistant to the antibiotics (Meropenem, Doripenem and Imipenem) whereas the non-capsulated strains were susceptible to the antibiotics. The results of this study however suggest the involvement of capsule in the resistance of the antibiotics employed.

Keywords: Capsule, *Pseudomonas aeruginosa,* Carbapenem, Antimicrobial interception

INTRODUCTION
Antimicrobial resistance by pathogenic bacteria has for long posed enormous turmoil in the fight against pathogenic diseases (Sabnis *et al.,* 2018; Blair *et al.,* 2015; Geisinger and Isberg, 2015; Sievert *et al.,* 2013; Chitnis *et al.,* 2012; Hidron *et al.,* 2008; Peleg *et al.,* 2008; Lockhart *et al.,* 2007). For decades, researchers across the globe have been trying to identify the various mechanisms by which microorganisms resist antimicrobials. Many of the pathogenic bacteria are enclosed by a capsular polysaccharide which is believed to be associated with virulence and antimicrobial interception (Sabnis *et al.,* 2018; Geisinger and Isberg, 2015; Jones *et al.,* 2009; Llobet *et al.,* 2008; Campos *et al.,* 2004). The degree of notoriety of many pathogenic bacteria have been linked to the presence of capsular polysaccharide (Sabnis *et al.,* 2018; Geisinger and Isberg, 2015).

The knowledge of antimicrobial interception by microorganisms has revolutionized the concept of antibiotic resistance. Antimicrobial interception literally involves the use of

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specialized molecules some of which are integral part of the microbial membrane and envelope to hinder antimicrobials from reaching their target (Sabnis *et al.,* 2018). Many act as decoys by mimicking the structural target of the antimicrobials thereby sequestering them, and creating a safe space for the pathogen (Sabnis *et al.,* 2018; Geisinger and Isberg, 2015).
Pseudomonas aeruginosa is a Gram-negative, aerobic/facultative anaerobic, encapsulated rod shaped bacterium that can cause diseases in plants and animals including humans (Qureshi, 2020; CDC, 2019). . It affects many parts of the body causing infections such as pneumonia, bacteremia, endocarditis, meningitis, brain abscess, otitis externa and media, bacterial keratitis, endophthalmitis, osteomyelitis, diarrhea, enteritis, enterocolitis, ecthyma gangrenosum and urinary tract infections among others (Diggle and Whiteley, 2020; Qureshi, 2020; CDC, 2019; CDC, 2014; Hoiby *et al.,* 2010; Diekema *et al.,* 1999; Fine *et al.,* 1996; Balcht and Smith, 1994;).

According to World Health Organization (WHO) (2017), in its news bulletin involving priority pathogens to which resistance have been developed to the first line drugs, and to which the urgent need for the search of new antibiotics against these pathogens, has listed *P.aeruginosa* among the first priority(critical) pathogens with resistance to carbapenem.
Only very limited information is available on the role of capsular polysaccharide of *P.aeruginosa* in antimicrobial resistance. This research however was conducted to give additional information on the role and mechanisms of capsular polysaccharide in antimicrobial interception and further provides an avenue and insight in tackling the problem of antimicrobial interception.

MATERIALS AND METHOD

Sample collection and isolation of *P. aeruginosa*

A total of 300 samples of swabs, urine, sputum, blood, stool and semen were collected from the pathology department of Murtala Muhammed Specialist Hospital and Aminu Kano Teaching Hospital for the isolation of *Pseudomonas aeruginosa*. The samples were primarily cultured on Cetrimide agar for the presumptive isolation of *P. aeruginosa.* Suspicious colonies were isolated and confirmed using biochemical and molecular techniques

Test for the presence of capsule

Indian ink staining technique was employed to test for the presence of capsule using the method described by Duguid (1951) and adopted by Geisinger and Isberg (2015).
Using a clean glass slide, a thin film of the bacterial cells was prepared using an Indian ink. The slide was allowed to air dry. It was flooded with crystal violet for one (1) minute and washed gently with distilled water not to avert distortion of the capsule. The slide was allowed to air dry and was subsequently viewed under oil immersion objective.

Antibiotic susceptibility test

This test was carried out according to the guidelines of CLSI (2018). An overnight culture of the test bacterium (turbidity equivalent to 0.5 McFarland) was streaked evenly on to the plates of Mueller Hinton agar. Commercially prepared Carbapenem discs (Meropenem 30µg, Imipenem 30µg and Doripenem 30µg) were placed on to the seeded agar at equal distance. The plates were incubated aerobically at 37^{o}C for 24 hours. After the incubation, zone of inhibition was observed and measured.

Determination of the minimum inhibitory concentration of the antibiotics

The minimum inhibitory concentration (MIC) was determined by ensuring that overnight culture of the test bacterium (turbidity equivalent to 0.5 McFarland) was inoculated in to five (5) set of tubes containing 5mL Mueller Hinton broth. Various concentrations of the antibiotics (10, 20, 30, 40 and 50 µg/mL) were prepared. One (1) mL of each dilution was added in to a tube containing the test bacterium and incubated aerobically at 37 °C for 24 hours. After the incubation, the emergence of turbidity was observed.

Determination of the minimum bactericidal concentration of the antibiotics

The minimum bactericidal concentration (MBC) of the antibiotics was determined by culturing a loopful from the above setup on to nutrient agar plates and incubated aerobically at 37 °C for 24 hours. Growth was observed at the end of the incubation.

RESULTS

P. aeruginosa was isolated from 49 (16%) out of 300 samples analyzed. Capsule was detected in 42 (86%) *P. aeruginosa* isolates while the remaining 7 (14%) did not show any visible capsule (Table 1). The capsule was observed as a clear halo around the bacterial cell after staining with India ink.

Antibiotic susceptibility test was carried out using disc diffusion method. All the capsulated strains were resistant to meropenem 30µg, doripenem 30µg, and imipenem 30µg. The strains where capsule was not detected showed a remarkable sensitivity to the antibiotics (Table 2). Only non-capsulated strains were inhibited by the antibiotics, the MIC was found to be 20 µg/mL for meropenem and imipenem and 10 µg/mL for doripenem. The MBC was detected at 40 µg/mL for meropenem and imipenem, 20 µg/mL for doripenem (Table 3).

Table 1: Morphological, biochemical and capsule characteristics of *P. aeruginosa* isolates

	Biochemical								Gram Stain	Morphology
	I	Ca	Co	M	Ci	O	U	V		
Capsulated (n=42)	-	+	-	-	+	+	-	-	-	rods
Non-Capsulated (n=7)	-	+	-	-	+	+	-	-	-	rods

Key: I=Indole, Ca= Catalase, Co=Coagulase, M=Methyl red, Ci=Citrate, O=Oxidase, U=Urease, V=Voges Proskauer, +=Positive, - =Negative

Table 2: Susceptibility of *P. aeruginosa* to Carbapenem (Meropenem, Doripenem and Imipenem)

Strains	Antibiotics		
	Meropenem 30µg	Doripenem 30µg	Imipenem 30µg
Capsulated	R	R	R
Non-Capsulated	S	S	S

Key: R=Resistant, S=Sensitive

Table 3: MIC and MBC values of the Antibiotics

Strains	MIC			MBC		
	Me	Do	Im	Me	Do	Im
Capsulated	Nil	Nil	Nil	Nil	Nil	Nil
Non- Capsulated	20 µg/mL	10 µg/mL	20µg/mL	40 µg/mL	20µg/mL	40 µg/mL

Key: Me=Meropenem, Do=Doripenem, Im=Imipenem, Nil=Not detected

DISCUSSION

Pseudomonas aeruginosa is a leading cause of infection in human and animals (Diggle and Whiteley, 2020; Qureshi, 2020; CDC, 2019; CDC, 2014; Hoiby *et al.,* 2010; Diekema *et al.,* 1999; Fine *et al.,* 1996; Balcht and Smith, 1994;). The bacterium is known for its high degree of notoriety with resistance to the last line of antibiotic which is carbapenem (WHO, 2017). A vast number of virulence factors and resistance mechanisms have been studied for this bacterium. Most of the well-studied resistance mechanisms in *Pseudomonas aeruginosa* include high efflux systems that pump antibiotics out of the cell, production of enzymes that inactivates antibiotics and restricted outer membrane permeability (Pang *et al*., 2019; Breidenstein *et al.,* 2011).

This current study however assessed the role of capsule of *Pseudomonas aeruginosa* in antibiotic resistance. Capsule was detected in 42 (86%) isolates of the bacterium. All the capsulated strains were resistant to the antibiotics whereas non-capsulated strains were susceptible to the antibiotics. This indicates that the capsule might have played a significant role in the antibiotic resistance. In a similar study conducted on *Acinetobacter baumanii* to determine the modulation of capsular polysaccharide in response to antibiotic exposure, the bacterium tended to increase the size of its capsule when exposed to antibiotics which was found to increase the survival of the bacterium (Geisinger and Isberg, 2015). The findings of this study are also in conformity with other studies that demonstrated the role of capsular polysaccharide in resistance to antimicrobial substances such as antibiotics and host immune defenses (Geisinger and Isberg, 2015; Sugawara and Nikaido, 2012; Coyne *et al.,* 2011; Cole *et al.,* 2010; Jones *et al.,* 2009; Zaragoza *et al.,* 2008; Llobet *et al.,* 2008; Spinosa *et al.,* 2007; Campos *et al.,* 2004; Nikaido, 2003).

CONCLUSION

The results generated from this study have demonstrated that capsule is used by this bacterium to resist antibiotics, as non-capsulated strains showed high level of sensitivity to the antibiotics employed. This research serves as a baseline for further studies which should focus on agents that disrupt the capsule of bacteria in its provision of an easy access to antimicrobial substances into bacterial cell.

ACKNOWLEDGEMENT

Big thanks to Tertiary Education Trust Fund (TETFund), Nigeria for the funding this work. Profound appreciation goes to the Research and Development unit of Kano University of Science and Technology Wudil (KUST) for the opportunity. We appreciate the Pathology Department of Aminu Kano Teaching Hospital and Murtala Muhammad Specialist Hospital for providing us with the bacterial isolates used in this study.

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