ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 10, Issue, 10, pp.10266-10271, October, 2019

RESEARCH ARTICLE

COMPARISON OF BIOFILM PRODUCTION AMONG CARBAPENEM RESISTANCE CLINICAL AND ENVIRONMENTAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* IN SOME TERTIARY HOSPITALS OF SOUTH WEST NIGERIA

[1,*]Ayilara A.O., [2]Oloke J.K. and [3]Olaitan J.O.

[1]Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology Teaching Hospital Osogbo, Osun State, Nigeria

[2]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo state, Nigeria

[3]Department of Biological Sciences, Osun State University, Osogbo, Osun State, Nigeria

ARTICLE INFO

Article History:
Received 25th July, 2019
Received in revised form
29th August, 2019
Accepted 27th September, 2019
Published online 30st October, 2019

Key words:

Pseudomonas aeruginosa, Carbapenen antibiotics, Carbapenem resistant, biofilm production, susceptibility testing,, nosocomial infection, community acquired infection, environmental *Pseudomonas aeruginosa*, clinical *Pseudomonas aeruginosa*, anitbiotic resistance.

****Corresponding author:***

ABSTRACT

Pseudomonas aeruginosa (P.A) is one of the major opportunistic pathogens of human often associated with morbidity and mortality. This study is to determine antibiotic susceptibility pattern and biofilm production among the P.A isolates and also compare biofilm production among carbapenem resistant clinical and environmental isolates from the selected Hospitals. Strains were recovered from different clinical and environmental samples from the hospitals using standard microbiological techniques. Kirby-Bauer disc diffusion method was used for susceptibility testing and biofilm production was determined using quantitative method. A total of 132 stains (120 clinical and 12 environmental isolates) of non duplicate P.A were recovered from 1,214 clinical and 1,000 environmental samples of the selected hospitals. Imipenem was the most effective antibiotics against the isolates as 84.2% (101) and 100% (12) were recorded from clinical and environmental isolates respectively. Biofilm production was 41.7% and 16.7% among clinical and environmental P.A and carbapenem resistant clinical P.A produces more biofilm than carbapenem resistant environmental P.A with 63.9% and 40% respectively. In conclusion policy maker and health practitioners should address this problem and implement a more rational and appropriate use of antibiotics. This may involve restricting and monitoring the sales of antibiotics in the country.

***Citation: Ayilara A.O., Oloke J.K. and Olaitan J.O.* 2019.** "Comparison of Biofilm Production among Carbapenem Resistance Clinical and Environmental Isolates of Pseudomonas aeruginosa in Some Tertiary Hospitals of South West Nigeria.", *Asian Journal of Science and Technology*, 10, (10), 10266-10271.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen from human that is frequently associated with an increased in morbidity and mortality among immunocompromised patients. (Kerr *et al* 2009, Armour *et al* 2007) Increasing resistance of *Pseudomonas aeruginosa* to different class of antibiotics especially carbapenem has become a major concern worldwide. Treatment of infection associated with *Pseudomonas aeruginosa* is usually complicated because the organism is intrinsically resistant to many classes of antibiotics and most times acquire resistant to most effective antibiotics (J.P. Pirnay *et al*, 2003). Abuse and continuous misuse of broad spectrum antibiotics has created selective pressure on the bacteria which has likely led to emergence of multi resistant strains. Research shows that multidrug resistant *Pseudomonas aeruginosa* is responsible for 4-60% nosocomial infections around the globe (H.G Safaei *et al*, 2017).

Pseudomonas aeruginosa is also known to produce wide arrays of virulence factors such as biofilm. The gravity of infection caused by *Pseudomonas aeruginosa* is dependent on the production of several virulence factors which affect different aspects of its pathogenesis (M.Y Alikhani *et al*,2014, M. Safari *et al* 2014 and J.A. Schaber *et al,* 2007,). Study also shows that the ability of *Pseudomonas aeruginosa* to form biofilm under different conditions reduces the efficacy of the antibiotics treatment and also induces chronic infectious diseases (T. Rasamiravaka *et al*, 2015).

Biofilms are sessile communities of microorganisms which are characterized by the cells being irreversibly attached to a substratum or to each other. They are enclosed in a matrix of extracellular polymeric substances (EPS) that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription (G.D Christensen *et al*, 1985). Biofilms act as efficient barriers against antimicrobial

agents and also help microorganism to attach to surfaces, protect them against drying and cause a lot of problems in the medical and industrial settings because of its ability to provoke greater resistance to antibiotics and also reduces the host immune response action (H.Grabski *et al,* 2017, T. Rasamiravaka *et al*, 2015 and M.E Davey *et al,* 2000). *Pseudomonas aeruginosa* is a worrisome organism that has the ability to grow under various conditions. The organism is found frequently in various areas of the hospitals such as physicians and nurses clothings, sinks, equipment, tap and contaminated medical equipment due to its high resistance to disinfectants (M. Davane *et al*, 2014 and De Abreu *et al,* 2014). The hospital environment may be contaminated through the hospital staff, patients and even visitors. It is very possible also for hospital area to harbour carbapenem resistant *Pseudomonas aeruginosa* and consequently circulates it within the hospital. In view of these, the objectives of this study is to isolate and identify *Pseudomonas aeruginosa* from clinical and environmental samples in the selected tertiary Hospitals, determine their antibiotic susceptibility pattern, determine production of biofilm among clinical and environmental *Pseudomonas aeruginosa* isolates and also compare biofilm production among carbapenem resistant clinical and environmental *Pseudomonas aeruginosa* from the selected Hospitals. Community acquired infections in this content are infections from the communities or infections acquired within 24hrs of admission in the hospital while nosocomial infection are refers to infections acquired after 24hours of admission into the hospital. Isolate found to be resistant to one out of the three carbapenem antibiotics applied was referred to as carbapenem resistant *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

The study was carried out in the Department of Medical Microbiology and Parasitology Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo from January 2017 to December 2017. A total of 1,214 clinical samples from wound swab, urine, stools, blood, sputum, ear swab and throat swab submitted to Microbiology Departments of two tertiary hospitals in South West Nigeria namely: LAUTECH Teaching Hospital-Osogbo and LAUTECH Teaching Hospital-Ogbomoso were examined. Also, 1,000 environmental samples recovered from beddings, sinks, furniture, equipment, and walls of the two tertiary hospitals were processed following standard microbiology procedures.

Identification of Pseudomonas aeruginosa: Preliminary isolation and identification of *Pseudomonas aeruginosa* was done by standard microbiology methods. Clinical Samples from wound swab, blood, urine, sputum, ear swab, throat swab and stool were cultured by streaking on MacConkey and chocolate agar plates with a sterile wire loop using standard procedures. The plates were incubated aerobically overnight at 37˚C. Environmental samples from the hospitals were also processed following standard microbiological procedures. Samples from sinks, equipment, beddings were collected using a sterile swab stick soaked with peptone water and were cultured immediately using a streak method with a sterile wire loop on chocolate and MacConkey agar. This was then incubated overnight at 37˚C. Isolates presumed to be *Pseudomonas aeruginosa* were identified by using colonial morphology, Gram reactions, motility test, biochemical tests, production of blue green pigment pyocyanin and also its ability to grow at 42^{O}C.

Antibiotics Susceptibility Testing: Antibiotic susceptibility test was determined for *Pseudomonas aeruginosa* isolates using Kirby-Baurer techniques on Mueller Hilton (MH) agar plates (Oxiod products). A sterile wire loop was used to pick test organism into a Mueller Hilton broth and was incubated at 37 ºC for 2 hours. Turbidity of the suspension was adjusted to 0.5 Macfarland's standard ($1.5x\ 10^8$ CFU/ml) and a sterile swab was then dipped into the inoculum tube which was used to streaked the entire surface of the MH agar to ensure even distribution. Antimicrobial disc was then picked using a sterile forceps and was pressed lightly to ensure contact with the agar which was then incubated at 37ºC for 18 hours. Antibiotic discs used include : ceftriaxone (CRO-10µg), ciprofloxacin (CIP-10µg), gentamycin (CN-10µg), tobramycin (TOB-10µg), levofloxacin LEV- 5µg), tetracycline (TE-30µg), imipenem (IPM-10µg), meropenem (MEM-10µg) and ertapenem (ETP-10µg) *Pseudomonas aeruginosa* strain ATCC 27853 was used to control the experiment. Zone diameter of inhibition (in mm) of the organism to each antibiotic were measured using a calibrated ruler put on the underside of the plate and was interpreted according to CLSI (2015).

Quantitative Determination of biofilm Production assay: Quantitative determination of biofilm assay was carried out using a sterile 96 well microtitre plate. *Pseudomonas aeruginosa* was first incubated overnight inside a Muller Hilton broth at 37°C. A volume of 100µl of the overnight cultures of *Pseudomonas aeruginosa* isolates were then inoculated into another 100 µl of Muller Hinton Broth (MHB) supplemented with 0.5% glucose for 2 days at 37°C. After incubation, the planktonic bacteria were removed by shaking the plate rapidly in a tray with tap water. 125 µl of 0.2% crystal violet was then used to stain the preparation for 10 minutes at room temperature before rinsing with distilled water. It was then air dried and 200 µl of 95% ethanol each was added to each well. Incubation was done at room temperature for 15 minutes and 150 µl from each well was transferred into a new microtitre plate. The absorbance was measured spectrophotometrically at 570 nm. The experiment was carried out in triplicates and the mean value of optical densities (OD) was calculated. The isolates with higher OD greater or equal to 0.2 were considered as biofilm forming isolates (A. Ghadaksaz *et al*, 2015 and J.H Merritt *et al*, 2005).

Patients information: Information about the age, sex, clinical details, sample type and date of collection of samples from the patients were collected from the laboratory forms filled for each patient by their physicians.

Inclusion criteria: All clinical and environmental samples cultured that yielded growth of *Pseudomonas aeruginosa* from the two hospitals were included.

Exclusion criteria: Any sample that does not grow *Pseudomonas aeruginosa* was excluded.

Preservation of isolate: Isolates were preserved in two slants in Mueller Hilton broth. One was preserved using 20% glycerol while the other was without gylycerol in the refrigeration at -20C in LAUTECH (Mercy Land) Osogbo before further investigation.

Data analysis: Data were generated from the results and SPSS 24 was used to analyze it. Results were generated through frequency and percentage.

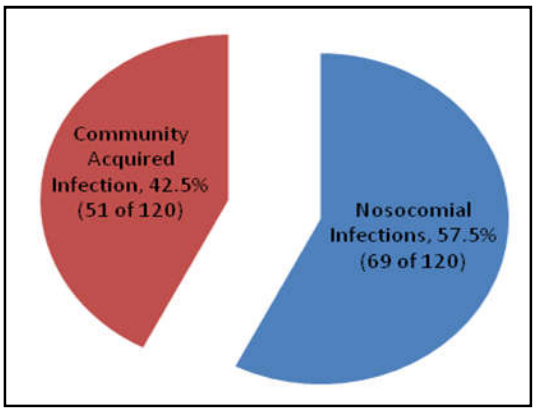

Figure 1. Distribution of *Pseudomonas aeruginosa* among Nosocomial and Community Acquired Infected Patients in the two Tertiary Hospitals of Southwest Nigeria (n=120)

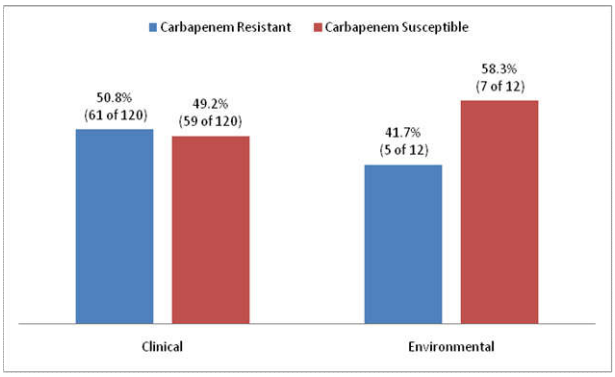

Figure 2. Carbapenem Resistant among Clinical and Environmental Isolates of *Pseudomonas aeruginosa* in the Selected Tertiary Hospitals

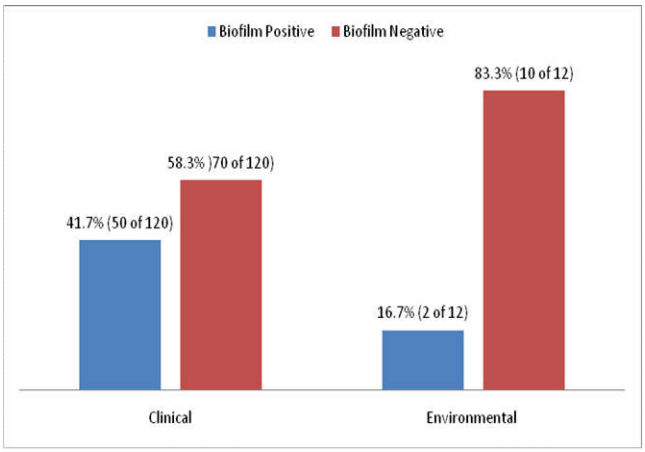

Figure 3. Distribution of Biofilm Production among Environmental and Clinical Isolates of *Pseudomonas aeruginosa* in Two Tertiary Hospitals of Southwest Nigeria. (Clinical *Pseudomonas aeruginosa* n=120, Environmental *Pseudomonas aeruginosa* n= 12)

***Ethical Approval*:** Ethical Approval was gotten from the selected hospitals.

RESULTS

A total number of 120 (9.9%) clinical and 12(1.2%) environmental isolates of non duplicate *Pseudomonas aeruginosa* were recovered from 1,214 clinical and 1,000 environmental samples respectively.

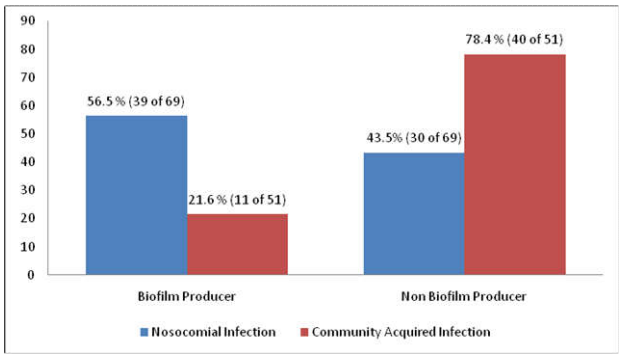

Figure 4. Biofilm Formation among Nosocomial and Community Acquired infected Patients of the Two Tertiary Hospitals in South West Nigeria

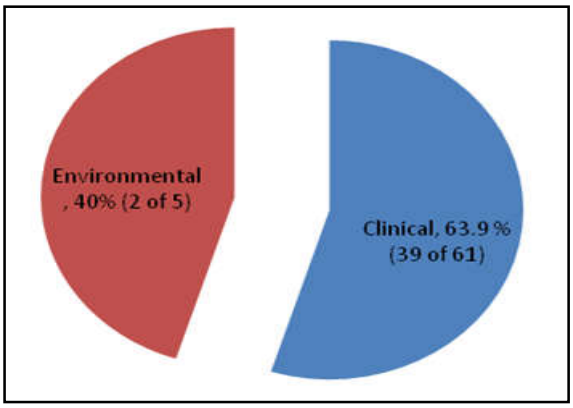

Figure 5. Evaluation of Biofilm Production among Carbapenem Resistant Clinical and Environmental *Pseudomonas aeruginosa*

Table 1: Gender Distribution of patients whose samples yielded growth of *Pseudomonas aeruginosa* among the two Tertiary Hospitals in Southwest Nigeria (n=120).

Gender	Frequency	Percentage (%)
Male	73	60.8
Female	47	39.2
Total	120	100

The patients from whom clinical *Pseudomonas aeruginosa* were isolated includes 73 male (60.8%) and 47 (39.2%) female (Table 1). Nosocomial infection was 69 (57.5%) while community acquired infection was 51(42.5%) (Figure 1) Age range of 31-40 have the highest frequency of 48(40%) while 51 and above was 3(2.5%), being the lowest (Table 2). Results of clinical and environmental *Pseudomonas aeruginosa* antimicrobial susceptibility testing by the disk diffusion method shows that Imipenem was the most effective antibiotics against the isolates as 84.2%(101 of 120) and 100% (12 of 12) were recorded for clinical and environmental isolates respectively. Highest resistance was seen among the clinical and environmental *Pseudomonas aeruginosa* against ceftriazone as 79.2% (95 of 120) and 75% (9 of 12) were resistant respectively. Other antibiotics exhibited various susceptibility rates as shown in Table 3. A proportion of 50.8% (61 out of 120) of clinical *Pseudomonas aeruginosa* were carbapenem resistant while 41.7% (5 out of 12) of Environmental *Pseudomonas aeruginosa* were carbapenem resistant (Figure 2). Biofilm formation among the clinical *Pseudomonas aeruginosa* was found to be 41.7% (50 out of 120) and 16.7% (2 out of 12) among environmental isolates (Figure 3).

Table 2. Age Distribution of Patients whose samples yielded growth of *Pseudomonas aeruginosa* in the Two Tertiary Hospitals of Southwest Nigeria (n=120).

Age Group (years)	Frequency	Percentage
1 0 - 10	11	9.2
11 - 20	14	11.7
21 - 30	35	29.1
31 - 40	48	40
41 - 50	9	7.5
51 and Above	3	2.5
Total	120	100

Table 3. Antibiogram of Clinical and Environmental *Pseudomonas aeruginosa* Isolates among the Two Tertiary Hospitals (Clinical isolate: n=120 , Environmental Isolates n=12)

Antibiotics	Clinical Isolates (n=120)				Environmental Isolate n=(12)			
	Sensitive		Resistant		Sensitive		Resistant	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
Ciprofloxacin	69	57.5	51	42.5	7	58.3	5	41.7
Gentimicin	44	36.7	76	63.3	5	41.7	7	58.3
Tobramycin	82	68.3	38	31.7	8	66.7	4	33.3
Levofloxacin	69	55.8	53	44.2	9	75	3	25
Ceftriazone	25	20.8	95	79.2	3	25	9	75
Imipenem	101	84.2	19	15.8	12	100	0	0
Meropenem	97	80.8	23	19.2	11	91.7	1	8.3
Etrapenem	59	49.2	61	50.8	7	58.3	5	41.7
Tetracycline	0	0	120	100	0	0	12	100

Table 4. Distribution of Biofilm Production among *Pseudomonas aeruginosa* from Different Clinical Samples in Some Tertiary Hospitals in South West Nigeria (n=120)

Clinical P.aeruginosa	Biofilm Producers	Non-Biofilm Producers
Sputum (N=16)	6 (37.5%)	10(62.5%)
Blood (N=15)	8 (53.3%)	7(46.7%)
Wound (N=43)	19 (44.2%)	24(55.8%)
Urine (N=36)	13 (36.1%)	23(63.9%)
Ear (N=10)	4 (40%)	6 (60%)

Table 5. Biofilm production among Clinical and Environmental Antibiotic Resistant *Pseudomonas aeruginosa*

Resistant Antibiotics	Clinical *Pseudomonas aeruginosa*			Environmental *Pseudomonas aeruginosa*		
	Number of Resistant Organism (n)	Biofilm Producers Frequency	%	Number of Resistant Organism	Biofilm Producers. Frequency	%
Ciprofloxacin	51	28	54.9	5	2	40
Gentimicin	76	42	55.3	7	1	14.3
Tobramycin	38	18	47.4	4	1	25
Levofloxacin	53	24	45.2	3	1	33.3
Ceftriazone	95	37	38.9	9	2	11.1
Imipenem	19	10	52.6	0	0	0
Meropenem	23	13	56.5	1	1	100
Etrapenem	61	39	63.9	5	2	40
Tetracycline	120	50	41.7	12	2	16.7

Maximum biofilm production was found among *Pseudomonas aeruginosa* isolates with 56.5% (39 out of 69) and 21.6% (11 out of 51) from nosocomial infection and community acquired infected patients respectively (Figure 4). This study shows that among the clinical samples cultured, blood has the maximum biofilm production with 53.3% (8 out of 15), followed by wound 44.2% (19 out of 43), ear 40% (4 out of 10), sputum 37.5% (6 out of 16) and urine 36.1%(13 of 36) (Table 4). Biofilm production among the resistant clinical and environmental *Pseudomonas aeruginosa* to different antibiotics shows maximum production among Etrapenem and meropenem with 63.9% (39 of 61) and 100% (1 of 1) respectively while other results are shown in Table 5. Biofilm production among carbapenem resistant clinical *Pseudomonas aeruginosa* was 63.9% (39 of 61) while among environmental it was 40% (2 of 5) Figure 5.

DISCUSSION

Infections caused by *Pseudomonas aeruginosa* are commonly implicated as a cause of health care acquired infections with high mortality and morbidity (N.A. Hassuna *et a*l 2015). Research shows that the ability of *Pseudomonas aeruginosa* to produce biofilms and to be resistant to multiple antibiotics allows the organism to survive any type of harsh conditions (De Almeida *et al*., 2017, P.K.Taylor *et al*., 2014). Major cause of the spread of this pathogen has been linked to cross transmission of *Pseudomonas aeruginosa* from health workers or inanimate objects in our health care institutions. Another major concern is the insufficient and inappropriate control of *Pseudomonas aeruginosa* infections which has led to spread of antibiotics resistant in our communities. (M.R. Arabestani *et al*, 2014, J.Rossello *et al*, 1992).

Formation of Biofilms by this pathogen does not only contribute to the resistance mechanisms against broad spectrum antibiotics but is also known to fight against host immune systems. Due to the restricted antibiotics penetration, adaptive response and presence of persisting cells, antibiotics susceptibility of biofilm producing bacteria is reduced (E. Drenkard *et al* 2003). Biofilm structure is recognised as a major factor for the persistence of several infections. Chronic infections, especially those associated with indwelling devices have been demonstrated to be involved in biofilm production (J.W. Costerton *et al*, 1999). Several authors have shown that antibiotic resistance of bacteria due to biofilm formation contribute to the persistence of bacterial cells and are also responsible for most problems encountered in the implementation of infection eradication programmes (A. Al-Ahmad *et al,* 2014, S. Gil-Perptin *et al*, 2012). In this study, isolation, identification, antibiotics susceptibility pattern and biofilm production of *Pseudomonas aeruginosa* from clinical and environmental samples in the selected tertiary Hospitals was determined. Production of biofilms among carbapenem resistant *Pseudomonas aeruginosa* was also determined among the clinical and environmental isolates from the selected Hospitals. The antimicrobial susceptibility profile among the 120 and 12 clinical and environmental *Pseudomonas aeruginosa* identified in this study shows high resistance to tetracycline, ceftriazone and Gentimicin with highest susceptibility to imipenem and meropenem among the clinical and environmental *Pseudomonas aeruginosa* (Table 3) Studies by some authors also shows high antibiotic resistance to tetracycline and gentimicin in both clinical and environment isolates (Z. Mohammed *et al*,2008, R.Ndip *et al*, 2005, L. Ruiz et al 2004). In Nigeria Zubair *et al*., 2018 reported 88% susceptibility to imipenem and 71.9% to meropenem among clinical *Pseudomonas aeruginosa*. This work corresponds with work done by Zubair *et al*. The high level of resistance experienced by some of the antibiotics applied could be due to the fact that most of the antibiotics are always available at the counters and had been highly misused overtime. (R.N. Akoachere *et al* 2002).

This work shows that clinical *Pseudomonas aeruginosa* is more resistant to carbapenem than environmental *Pseudomonas aeruginosa* with 50.8% (61) and 41.7% (5) respectively. A work done in Nigeria by Ayilara *et al.,* 2019 shows that clinical *Pseudomonas aeruginosa* are more resistant to carbapenem than environmental *Pseudomonas aeruginosa* with 28.5% and 10% respectively (A.O Ayilara *et al.,* 2019). Even though this present reports show a high percentage of resistant to carbapenem than the previous work done, this different may be due to the fact that different brand of carbapenem were used in the two research. This shows that resistant of *Pseudomonas aeruginosa* to carbapenen antibiotics may likely depend on the brand of carbapenem used. Another report by Ruiz *et al*., shows that clinical isolates are more resistant to antimicrobial agents than environmental isolates which may be caused by constant submission of clinical strains to the selective action of antibiotics (L. Ruiz et al 2004). Biofilm production among the nosocomial infected and community acquired infected patients was 56.5% and 21.6%. This study shows that there was no association between biofilm production and duration of stay of patients in the hospitals. Blood samples was found to have maximum biofilm production among the isolates followed by wound samples this contradicted what was done by Swarnatrisha Saha and his group in Indian in which cathether tip was found to have the highest level of biofilm production.(Swarnatrisha *et al*, 2018). There was no association between biofilm production and the type of samples collected. This results shows that biofilm production was higher among carbapenem resistant clinical *Pseudomonas aeruginosa* than carbapenem resistant environmental *Pseudomonas aeruginosa* (Figure 5). Previous work by Ghanbarzdeh et al., and Abidi *et al.,* shows statistically biofilm formation in the MDR *Pseudomonas aeruginosa* was higher than the non multi drug resistant *Pseudomonas aeruginosa* (Z. Ghanbarzadeh *et al*., 2015 and S.H. Abidi *et al*, 2013). All efforts to lay hands on previous work done on biofilm production among carbapenem resistant *Pseudomonas aeruginosa* clinical and environmental isolates proves abortive. In conclusion a high prevalence of antibiotic resistant and high biofilm production was observed in both the clinical and environmental isolates of *Pseudomonas aeruginosa*. It was also observed that biofilm production was higher among carbapenem resistant *Pseudomonas aeruginosa* than carbapenem resistant environmental *Pseudomonas aeruginosa* . This situation needs urgent attention in our health care setting. It is in our opinion that policy maker and health practitioners should address this problem and implement a more rational and appropriate use of antibiotics. This may involve restricting and monitoring the sales of antibiotics in the country.

Acknowledgements: Sincere appreciation goes to all the staff of Medical Microbiology and Parasitology Department, Ladoke Akintola Unversity of Technology Teaching Hospital Osogbo for their support in performing the laboratory investigations.

REFERENCES

Abidi, S.H. S.K Sherwani, T.R Siddiqui, A Bashir, S.U Kazmi. 2013. Drug resistance profile and biofilm forming potential of *Pseudomonas aeruginosa* isolated from contact lenses in Karachi-Pakistan. BMC Ophthalmol.13: Article number 57.

Al-Ahmad, A., H. Ameen, K.Pelz, L. Karygianni, A. Wittmer, A.C Anderson, et al. 2014. Antibiotic resistance and capacity for biofilm formation of different bacteria isolated from endodontic infections associated with root-filled teeth. *J Endod*. 1;40(2): pp 223-30.

Alikhani, M Y., Tabar, Z.K., Mihani, F., Kalantar, E., P. Karami, M. Sadeghi, S.A. khosroshahi, S. Farajnia. (2014) Antimicrobial resistance patterns and prevalence of blaPER-1 and blaVEB-1 genes among ESBL-producing *Pseudomonas aeruginosa* isolates in West of Iran. *Jundishapur J. Microbiol*: 7(1): e8888

Arabestani, M.R., Karami, M., Alikhani, M.Y. 2014. Antimicrobial resistance in microorganisms. *Avicenna J. Clin. Microbiol. Infect.*: e19941.

Armour, A.D., Shankowsky, H.A., Swanson T., Lee J. and Tredget. E.E. 2007. The impact of nosocomially-acquired resistant Pseudomonas aeruginosainfection in a burn unit. J. Trauma.63: pp 164–171

Ayilara, A.O., J.K Oloke., J.O Olaitan and M.A Muibi 2019. Comparison of Carbapenem Resistance among Clinical and Environmental *Pseudomonas aeruginosa* in South West Nigeria, International Journal of Recent Scientific Research Vol. 10, pp. 33863-33867.

Christensen, G.D., W.A. Simpson, J.A. Younger, L.M. Baddour, F.F. Barrett, D.M. Melton, et al. 1985. Adherence of cogulase negative Staphylococi to plastic tissue cultures:

a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol* ; 22: pp 996-1006.

Costerton, J.W., P.S Stewart, E.P Greenberg. 1999. Bacterial biofilms: a common cause of persistent infections. *Science*. 21;284(5418):pp 1318-22.

Davane, M., N. Suryawanshi , A. Pichare., B.S. Nagoba 2014. *Pseudomonas aeruginosa* from hospital environment, *J. Microbiol. Infect. Dis.,* : 4(1) : pp 42-43

Davey, M.E., G .A O'Toole. 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev; 64(4): pp 847–67.

De Abreu, P.M., Farias, P.G., Paiva, G.S., Almeida, A.M., Morais. P.V. 2014. Persistence of microbial communities including *Pseudomonas aeruginosa* in a hospital environment: a potential health hazard. *BMC Microbiol*., pp. 118

De Almeida Silva, K.C.F., Calomino, M.A., Deutsch, G.,. De Castilho, S.R., De Paula, G.R., Espe L.M.R., R, L.A Teixeira. 2017. Molecular characterization of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolated in a burn center Burns, 43, pp. 137-143

Drenkard E. 2003. Antimicrobial resistance of Pseudomonas aeruginosa biofilms. *Microbes Infect*. 1;5(13): pp 1213-9

FTK Akoachere, J. R.N Ndip, E.B Chenwi, L.M Ndip, Njock TE&Anong DN. 2002. Antibacterial effect of Zinziben officinale and R,N.Ndip et al Garcinia kola on respiratory tract pathogens, *East African Medical Journal* 79, pp 588-591.

Ghadaksaz, A., Fooladi, A.A., Hosseini, H.M., Amin, M. 2105. The prevalence of some Pseudomonas virulence genes related to biofilm formation and alginate production among clinical isolates. *Journal of Applied Biomedicine*. 28;13(1): pp 61-8.

Ghanbarzadeh Corehtash Z, Khorshidi A, Firoozeh F, Akbari H, Mahmoudi Aznaveh A. (2015;) Biofilm formation and virulence factors among *Pseudomonas aeruginosa* isolated from burn patients. Jundishapur J Microbiol. 8:e22345

Gil-Perotin, S., P. Ramirez, V.Marti, J .M Sahuquillo, E. Gonzalez,Calleja et al. 2012. Implications of endotracheal tube biofilm in ventilator –associated pneumonia response: a state of concept. *Critical Care*. *16*, Article number R93: http://ccforum.com/content/16/3/R93

Grabski, H., L .Hunanyan , S. Tiratsuyan, H. Vardapetyan 2017. Interaction of N-3-oxododecanoyl homoserine lactone with LasR protein of *Pseudomonas aeruginosa*: *insights from molecular docking and dynamics simulations. bioRxiv* , doi: https://doi.org/10.1101/121681

Hassuna, N.A., Mohamed, A.H.I., Abo-eleuoon, S.M., Rizk, H.AW.A 2015. High prevalence of multidrug resistant *Pseudomonas aeruginosa* recovered from infected burn wounds in children. *Archives of Clinical . Microbiology* . Vol 6 No 4: 1.

Kerr K.G. and Snelling A.M. 2009. Pseudomonas aeruginosa: a formidable and ever-present adversary *J. Hosp.Infec*., pp 338–344

M.Safari , M.Y. Alikhani, M.R. Arabestani, R.K. kakhki, R. Jafari (2014). Prevalence of Metallo-β-lactamases encoding genes among *Pseudomonas aeruginosa* strains isolated from the bedridden patients in the intensive care units. *Avicenna J. Clin. Microbiol. Infect*.

Merritt, J.H., Kadouri, D.E., O'Toole, G.A. 2005. Growing and analyzing static biofilms. Current protocols in microbiology.vol 00,issue 1. https://doi.org/10.1002 /978047 1729259.mc01b01s00

Mohammad Taheri, Z. N. Shahbazi, M .Khoddami. 2008. Genetic Diversity of *Pseudomonas aeruginosa* Strains isolated from Hospitalized patients. *Tanaffo*s; 7: pp, 32–9.

Ndip, R., H. Dilonga, L .Ndip., .J .Akoachere, T. Nkuo. Akenji., 2005. *Pseudomonas aeruginosa* isolates recovered from clinical and environmental samples in Buea, Cameroon: current status on biotyping and antibiogram**.** *Trop. Med. Int. Health*, pp. 74-81

Pirnay, J.P., De Vos, D., Cochez, C., Bilocq F., Pirson, J., Struelens M. et al. 2003. Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. *J Clin Microbiol.*, 41(3), pp 1192–202.

Rasamiravaka, T., Vandeputte, O.M., Pottier, L., Huet, J., Rabemanantsoa, C. Kiendrebeogo, M., Andriantsimahavandy, A., Rasamindrakotroka, A., Stévigny, C. and Duez. P. 2015. *Pseudomonas aeruginosa* biofilm formation and persistence, along with the production of quorum sensing-dependent virulence factors, are disrupted by a triterpenoid coumarate ester isolated from Dalbergia trichocarpa, a tropical legume. *PloS One., Article e0132791*

Rossello J. *et al* 1992. Investigation of an Outbreak of nosocomial infection due to multiple drugs resistant strains of Pseudomonas aeruginosa '*Journal of Hospital Infection* : Vol 20. Issue 2, pp 87-96

Ruiz, L. M.A Dominguez, N .Ruiz, M. Vinas. 2004. Relationship between clinical and environmental isolates of *Pseudomonas aeruginosa* in a hospital setting. *Arch Med Res*.;35: pp 251–7.

Safaei, H.G., Moghim, S., B.N. Isfahani, H. Fazeli, F. Poursina, S. Yadegari, P. Nasirmoghadas, Nodoushan, S.A.H. 2017. Distribution of the strains of multidrug-resistant, extensively drug-resistant, and pandrug-resistant *Pseudomonas aeruginosa* isolates from burn patients. Adv. *Biomed Re., 6 pp 156*

Schaber, J.A., Hammond, A., Carty, N.L., Williams, S.C., Colmer-hamood, J.A., Burrowes, B.H., Dhevan, V., Griswold, J.A. and Hamood, A.N. 2007. Diversity of biofilms produced by quorum-sensing-deficient clinical isolates of *Pseudomonas aeruginosa. J. Med. Microbiol.,* 56 pp. 738-748

Swarnatrisha Saha, Ksh Mamta Devi, Shan Damrolien, Kh. Sulochana Devi, Krossnunpuii, Kongbrailatpam Tharbendra (2018); Biofilm production and its correlation with antibiotic resistance pattern among clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital in north-east India Sharma International Journal of Advances in Medicine *Int J Adv Med*.;5(4): pp 964-968.

Taylor, P.K., Yeung, A.T., Hancock. R.E. 2014. Antibiotic resistance in *Pseudomonas aeruginosa* biofilms: towards the development of novel anti-biofilm therapies. *J. Biotechnol*,: 191 , pp. 121-130

Zubai K.O and K.C Iregbu. 2018. Resistance Pattern and Detection of Metallo-beta-lactamase Genes in Clinical Isolates of *Pseudomonas aeruginosa* in a Central Nigeria Tertiary Hospital. J ClinPract; 21:176-82.

****