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INCIDENCE OF COLISTIN RESISTANT BACTERIA IN OUT PATIENTS OF A GOVERNMENT HOSPITAL IN DELTA STATE, NIGERIA

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ABSTRACT

This study was carried out to determine the incidence of colistin resistance in hospitalized patients. Ten samples of high vaginal swap and twenty samples of urine were collected from out patients ofgeneral hospital, Agbor, Delta State. The organisms isolated from the samples included the following; *Enterococcus faecalis, Staphylococcus aureus, Bacillus Subtillis, Staphylcoccus epidermidis, Bacillus cereus, Streptococcuspyogene, Pseudomonas aeruginosa, Eschericha coli, Enterobacteraerogene*and *Neisseria gonorrhoeae.* Sensitivity test was carried out on the isolates obtained after morphological and biochemical tests had been carried out. The percentage prevalence of test isolate from urine sample showed that out of a total of 12 isolates, *Staphylococcus sp* had the highest percentage prevalence of 58.3% while *Entrococcusfaecalis* had the least percentage prevalence of 8.3% whereas the percentage prevalence of the isolates from high vaginal swap showed that *Staphylococcus* sp. had the highest percentage prevalence of 35% while *Pseudomonas aeruginosa*, Neisseria gonorrhea and *Enterobateraerogene* had the least percentage prevalence of 5% each. A high detection rate of colistin resistance among isolates was oserved in this study. All gram negative isolate were resistant to colistin. The increasing trend in antibiotic resistance especially to last resort drug as colistin continues to threaten global health and this is worrisome.

KEYWORDS: Colistin resistance, gram positive, gram negative, urine, high vagina swab.

1.0 INTRODUCTION

Colistin also known as polymyxin E is produced by *Paenibacilluspolymyxa*and belongs to the polymyxin class of antibiotics. It is a polycationic peptide with both hydrophilic and lipophilic domains. The cationic regions interact with bacterial lipopolysaccharide (LPS) in the outer cell membrane, where it displaces magnesium and calcium counter ions. Colistin is a multicomponent polypeptide antibiotic, comprised mainly of colistin A and B, colistin became available for clinical use in the 1960s, but it was replaced in the 1970s by antibiotics considered less toxic (Livermore *et al.*, 2004). There are two forms of colistin commercially available, they are: colistin sulphate for oral and tropical use, and colistin methane sodium (Sodium colistin methanesulphonate, colistinsulfomethane sodium) for parenteral use; both can be delivered by inhalation. Although there have been asubstantial number of clinical reports on the successful use of colistin (Suke*et al.*, 2005)or polymyxin B (Tolaney*et al.*, 2005) (which differs by only one amino acid from colistin) against infections cause by multidrug-resistant *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae*, there is a dearth of information on the clinical pharmacokinetics, pharmacodynamics, and toxicodynamics of colistin; such data are essential for establishing optima dose regiments (Turnidge *et al.*, 2005).

It has long been recognized that hospitalized patients are susceptible to infection, such as respiratory tract infections due to mechanical ventilation. Many of these infections are caused by aerobic Gram-negative bacteria originating from the digestive tract. These nosocomial infections can increase morbidity, mortality and healthcare costs (Troulle*et al.*, 2012). Antimicrobial resistance against colistin has emerged worldwide threatening the efficacy of one of the last-resort antimicrobials used for the treatment of infections caused by multidrug resistant bacteria The risk of acquiring multi drug resistant bacteria in hospitals is increase by severity of illness, length of stay, use of intravascular devices exposure of hospitalized patients to invasive therapeutic procedures like endotracheal intubation, the intensity of exposure to infected patients and the frequent

misuse of antibiotic drug (Akortha and Egbule (2008); Khan *et al.,* 2014; Egbule 2016; Egbule *et al*., 2016a; Egbule *et al*., 2016aKhan *et al.,* 2014).

Most knowledge on the pharmacokinetics of colstin was obtained at least two decades ago when non-specific microbiological assays were used to measure the concentration of "colistin" in biological fluids (Milne *et al.,* 2005). Colistin use was largely abandoned soon after its introduction in favor of aminoglycosides, which have a more favourableside effect profile as well as improved efficacy. However, the use of colistin has increased again in the last decade due to the emergence of carbapenem-resistant pathogens, including carbapeenem-resistant Entrrobacteriaceae (CRE) (Iweriebor, et al., 2022; Giannella *et al.,* 2013).

Colistin has become the single most important agent in the treatment of carbapenem-resistant Enterobacteriaceae (CRE) infections, and it is often used in combination with one or more other agents. Although widely used in the literature the term colistin and colistimethane are not interchangeable (Coulthard *et al.,* 2001). Colistin (Usually used as the suphate salt) is a polycation, whereas colistimethane (used as the sodium salt) is a polyanion at physiological pH. Colistin-methane is prepared from colistin by reaction of the free γ-amino groups of the five α, γ-diaminobutyric acid residues with formaldehyde followed by sodium bisulphite (Barnett *et al.,* 1964)

Colitin has been recently considered as last option treatment for patients with nosocomial pan drug resistant (PDR) infections, which have become an important public health issue, owing to its favourable properties of rapid bacterial killing, a narrow spectrum of activity and slow development of resistance (Falagas*et al.,* 2006). Colistin is commonly used in agriculture in many countries for the control of infections in pigs and, in particular, cattle (Egbule and Egbule 2015; Egbule and Yusuf 2019; Egbule et al., 2020; Iweriebor, et al., 2021; Iweriebor, et al., 2022). Its usage in humans was so far, rather, limited, mainly because of its renal toxicity, but it has been recently re-introduced as a last-line option to treat extensively antibiotic-resistant bacteria such as carbapenem resistant strains [i.e NDM-1 and KPC positive bacterial isolates or extensively drug-resistant (XDR). *Acinetobacterbaumanni* and *Pseudomonas aeruginosa*. Colistin is not homogeneous at the molecular level and consists of a mix of the cyclic polypeptides A and B. Colistin essentially solubilizes the bacterial cell membrane, which is bacteriacidal in an aqueous environment. It is an older drug with significant nephrotoxicity.

Increasing resistance of Gram positive and negative bacteria and emergence of colistin resistance is the threat for this last-resort antibiotic. Therefore, in this study, we investigated the incidence of colistin resistance in bacterial isolates recovered from hospitalized patients.

MATERIALS AND METHODS

Sample collection

Clinical samples comprising of high vaginal swab (HVS) and urine were collected from out patients attending General hospital at Agbor, Delta State by the help of a medical practitioner. Questionnaire containing information on the use and efficacy of colistin were also distributed to patient at the hospital.

Isolation of organism

The urine sample was centrifuge using the bucket centrifuge at 1500 revolution/minute for 15 minutes. This separated the urine into two distinct part, the supernatant and the pellet, the supernatantwas discarded while the pellet was used for culture. Few drops of the pellet sample were placed in the Cysteine lactose electrolyte deficient agar medium and incubated at 37^{o}C for 24 hours. A sterile wire loop was used to inoculate from the medium into another freshlyprepared Cysteine lactose electrolyte deficient agar medium. The plates were incubated at 37^{o}C and thereafter observed for obvious growth on the surface of the culture plate. Subsequent sub-culturing in selected media was carried out to further purifythe isolates. Culture were Gram-stained and morphologies of the organisms observed under the microscope.

Microscopy examination was first carried out on the high Vaginal Swab (HVS) specimen to check for pus cells, epithelia cells yeast etc. Few drops of the sample were place in the nutrient agar and MacConkey agar and incubated at 37^{o}C for 24 hours. A sterile wire loop was used to inoculate from the nutrient agar and MacConkey into nutrient and MacConkey agar plates. The plates were incubated at 37^{o}C for 24 hours and thereafter observed for obvious microbial growth on the surface of the culture plate. Subsequent sub-culturing in selected media was carried out to further purify the isolates.

Morphological and biochemical Identification of isolates

Microbiological investigation of bacteria isolates was conducted, cultures were Gram-stained and morphologies of the organisms observed under the microscope, biochemicaltest include motility test, catalase test, citrate utilization, indole production, ureasetest, glucose, lactose, hydrogen sulphide, acid, gas and oxidase test. The 2012 Bergey's Manual Volume 5 of Determinative Bacteriology was used for the identification.

RESULTS

The identification of isolate using several biochemical characterization and morphological characterization is presented in Table I. The organism isolated from the samples include the following; *Enterococcus faecalis, Staphylococcus aureus, Bacillus Subtillis, Staphylcoccus epidermidis, Bacillus cereus, Streptococcus pyogene, Pseudomonas aeruginosa, Eschericha coli, Enterobacter aerogene* and *Neisseria gonorrhoeae.*

Table 1: Identification of Bacteria isolate.

	Shape	Gram stain	Catalase	Oxidase	Iodine	Citrate	Lactose	Glucose	H^2S	Acid	Gas	Motility	
HVS	**Cocci**	+	+	-	-	+	+	+	-	-	-	-	***Staphylopcoccus aureus***
	Rods	-	+	-	-	+	+	+	+	-	-	+	***Enterobacter aerogenes***
	Cocci	+	-	-	-	-	+	+	-	-	-	-	***Enterococcus faecalis***
	Rods	-	+	-	+	+	-	+	-	+	+	-	***Escherichia coli***
	Rods	+	+	-	-	+	+	+	-	-	-	+	***Bacillus cerus***
	Rods	-	+	+	-	+	-	-	-	-	-	+	***Pseudomonas aeruginosa***
	Rods	+	+	-	-	+	+	+	-	-	-	+	***Bacillus subtilis***
	Cocci	-	+	+	-	+	+	+	+	+	-	-	***Neisseria gonorrhoeae***
	Cocci	+	-	-	-	-	+	+	-	-	-	-	***Streptococcus pyogene***
Urine	**Cocci**	+	-	-	-	-	+	+	-	-	-	-	***Enterococcus faecalis***
	Cocci	+	+	-	-	-	+	+	-	-	-	-	***Staphylopcoccus aureus***
	Rods	-	+	-	-	-	+	+	-	+	+	-	***Escherichia coli***
	Rods	+	+	-	+	+	+	+	-	-	-	+	***Bacillus subtilis***
	Cocci	+	+	-	+	-	+	+	+	-	+	-	***Staphylopcoccus*epidermidi**

The percentage prevalence of test isolate from urine sample in Table 2. Out of a total of 12 isolates, *Staphylococcus sp*. Had the highest percentage prevalence of 58.3% while *Entrococcusfaecalis* had the least percentage prevalence of 8.3%.

Table 2: Percentage prevalence of isolates from urine sample.

Bacterial Isolates	Number of isolate	Percentage Prevalence (%)
Staphylococcus sp	7	58.3%
Escherichia coli	2	16.7%
Bacillus subtilis	2	16.7%
Enterococcus faecalis	1	8.3%
Total	12	100%

The percentage prevalence of the isolates from HVRsample is showed in Table 3. It was observedthat *Staphylococcus* sp. Had the highest percentage prevalence of 35% while *Pseudomonas aeruginosa*, *Neisseria gonorrhea* and *Enterobateraerogene* had the least percentage prevalence of 5% each.

Table 3: Percentage prevalence of isolate from HVS sample.

Bacterial Isolates	Number of isolate	Percentage Prevalence (%)
Staphylococcus sp	7	35%
Escherichia Coli	2	10%
Bacillus sp	5`	25%
Enterococcus faecalis	2	10%
Pseudomonas auruginosa	1	5%
Neisseragonorrhoeae	1	5%
Streptococcus pyogene	1	5%
Enterobacter aerogene	1	5%
Total	20	100%

The Sensitivity profile of Gram positive isolate obtained from urine is presented as Table 4. The isolates were tested with a number of of antibiotics. Majority of the isolates were found to be sensitive to the texted antibiotics.

Table 4: Sensitivity profile of Gram Positive Isolates from Urine.

Isolate	CPX	E	LEV	CN	APX	RD	AMX	S	NB	CH
Staphylococcus sp	S	S	S	S	S	S	S	S	S	S
Staphylococcus sp	S	S	S	S	S	S	S	S	S	S
Staphylococcus sp	S	S	S	S	R	S	R	S	S	S
Staphylococcus sp	S	S	S	S	S	S	S	S	S	S
Staphylococcus sp	S	S	S	S	S	S	S	S	S	S
Bacillus subtilis	S	S	S	S	R	S	S	S	S	S
Staphylococcus sp	S	S	S	S	R	S	S	S	S	S
Bacillus subtilis	S	S	S	S	S	S	S	S	S	S
Staphylococcus sp	S	S	S	S	S	S	S	S	S	S
Staphylococcus sp	S	S	S	S	S	S	S	S	S	S

KEY
R:ResistanceS: SensitiveCPX: CiprofloxacinE: ErythromycinLEV: Levofloxacin
CN: GentamycinAPX: AmpicillinRD: RifampicinAMX: AmoxicillinS: Streptomycin
NB: NorfloxacinCH: Chloramphenicol

Sensitivity profile of gram-positive isolates obtained from high virginals swab is presented as Table 5, the result showed that gram-positive isolates obtained from high virginals swab were most resistant to Norfloxacin.

Table 5: Sensitivity profile of gram positive bacterial isolate from High Vaginal swab.

Isolate	**CPX**	**E**	**LEV**	**CN**	**APX**	**RD**	**AMX**	**S**	**NB**	**CH**
Bacillus cereus	S	S	S	R	S	S	S	S	R	R
Bacillus cereus	S	S	S	S	S	S	S	S	R	S
Staphylococcus aureus	S	S	S	R	S	S	S	S	S	S
Bacillus subtilis	S	S	S	S	S	S	S	S	S	S
Bacillus subtilis	S	S	S	R	R	S	R	S	R	S
Steptococcuspyogene	S	S	S	R	S	S	R	S	R	S
Bacillus subtilis	S	S	S	S	S	S	R	S	R	R
Staphylococcus aureus	S	S	S	R	S	S	S	S	R	S
Staphylococcus aureus	S	S	S	S	S	S	R	S	R	S
Staphylococcus aureus	S	S	S	R	S	S	S	S	S	S
Staphylococcus aureus	S	S	S	S	R	S	S	S	R	R
Staphylococcus aureus	S	S	S	R	S	S	R	S	R	R
Staphylococcus aureus	S	S	S	S	S	S	R	S	R	R
Enterococcus faecalis	S	S	S	R	R	S	S	S	R	S
Enterococcus faecalis	R	R	S	R	R	S	R	S	R	S

KEY
R:Resistance S: Sensitive CPX: Ciprofloxacin E: Erythromycin LEV: Levofloxacin
CN: Gentamycin APX: Ampicillin RD: Rifampicin AMX: Amoxicillin S: Streptomycin
NB: Norfloxacin CH: Chloramphenicol

Sensitivity profile of gram-negative isolates obtained from urine is presented in Table 6. E.coli was the only gram negative isolate obtained and was observed to be resistant to colistin and soe other antibiotics.

Table 6: Sensitivity profile of Gram Negative Isolate from Urine.

Isolate	**OFX**	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**	**C**
Escherichia coli	*S*	S	S	R	R	S	S	S	R	S	R

KEY
OFX: Tarivid, PEF: Pefloxacin, CPX: Ciprofloxacin, AU: Augmentin, CN: Gentamycin
S: Streptomycin, CEP: Ceprorex, NA: Nalidixic acid, SXT: Trimethoprim sulfamethoxazole
PN: Ampicilin, C: Colistin

Sensitivity profile of gram-negative isolates obtainedfrom high vaginal swab is presented as Table 7, isolate obtained from high vaginal swab which include *Pseudomonas aeruginosa, Neisseria gonorrhea, Escherichia coli*and *Enterobacter aerogenes*were all resistant to colistin.

Table 7: Sensitivity profile of Gram Negative Isolate from high vaginal swab.

Isolate	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN	C
Pseudomonas aeruginosa	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	R	R	R
Neisseria gonorrhea	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	R
Escherichia coli	*S*	*S*	*S*	*S*	R	R	R	*S*	R	R	R
Enterobacter aerogenes	*S*	*S*	*S*	*S*	R	R	R	R	R	R	R
Escherichia coli	*S*	*S*	*S*	*S*	*S*	*S*	R	*S*	*S*	*S*	R

KEY
OFX: Tarivid, PEF: Pefloxacin, CPX: Ciprofloxacin, AU: Augmentin, CN: Gentamycin
S: Streptomycin, CEP: Ceprorex, NA: Nalidixic acid, SXT: Trimethoprim sulfamethoxazole
PN: Ampicilin, C: Colistin

DISCUSSION

Despite growing information on the global spread and distribution few studies have looked into the prevalence of colistin resistantbacteria in African countries. Our study shows that humans colistin resistant bacteria are prevalent among patients in Nigeria. Colistin is an antibiotic produced by *Paenibacciluspolymyxa* and it is used in the treatment of infections caused by multidrug-resistance *Pseudomonas aeruginomas*, *Acinettobacterbaumannii*, Escherichia coli and *Klebsiellapneumonas*. A recent study revealed that patient colonizedby colonized by colistin-resistant isolates had previous colistin exposure compared to those who did not. (Kontopidou*et al.*, 2011).

A total of ten virginal swabs and a total of twenty urine samples were collected from both male and female patients. The bacteria isolated were *Staphylococcus aureus, Eschericha coli, Bacillus cereus, Enterococcus faecalis, Psedomonasaeruginos, Neisseria gonorrhoeae, Entrobactrtaerogen, Streptomonaspyogne, staphycococcus epidermidis* and *Baccillus subtilis.* The growing threat of colistin resistance may be due to the increases exposure of patients to colistin. This increase exposure to colistin may be because of the increased level of resistance associated with carbapenem (Li, et al., 2006). This has led to an increase in the use of colistin, an antibiotic previously discarded due to its high rate of toxicity, to treat these patients. As might be expected, the increase use of colistin has resulted in the emergence of pathogens that are resistant to this agent due to selective pressure, although some infections with colistin-resistant *Escherichia coli* has occurred without prior colistin use (Chen et al., 2011).

The high detection rate of colistin resistance among isolates in this study is consistent with reports from various part of the world **(**Nabti et al., 2019; Anyanwu et al., 2020; Bonnin et al., 2020). In agreement with previous studies, the majority of the positive isolates expressed high colistin resistance (Bonnin et al., 2020; Wang et al., 2019).

From the questionnaire distributed to patient in the hospital, the following results were obtained. A total of 50 questionnaires were distributed, amongst which 30 of them indicated that the colistin drug was not effective in the treatment of infections, while about 10 patients were not sure of colistin's effect in treating infections, the remaining patients had not come across the colistin drug before. The results obtained from the questionnaire further confirms the result obtained in the laboratory that colistin drug is not effective against infections.

The mechanism(s) of colistin resistance in carbapenem-resistance*Enterobacteriaceae* are not fully understood. The mechanism of colistin resistance in *Acinetobacterbaumannii* has been elucidated but it remains unclear whether the same mechanism is associated with colistin resistance in carbapenem-resistant is *Entrobacteriaceae*. Multiple co-morbidities, invasive procedures and most importantly, prior exposure to colistin, are likely to be the driving factors fueling the threat posed by colistin-resistant Enterobactericae. Pressure from colistin use not only exposed the recipients to infections with colistin-resistant organism, but it also led to resistance in other antibiotics as observed in this study. Isolates resistant to colistin were also found to be resistant to other antibiotics (Tables 6 and 7).

CONCLUSION

Colistin has been extensively used to treat Multi drug resistant Gram-negative bacteria, and this might lead to the development of resistance towards this agent.

The increasing trend in antibiotic resistance continues to threaten global health due to the limited spread of new antibiotics. To control this trend, a multidisciplinary approach that involves infectious disease pharmacist must be involved in the fight.

REFERENCES

1. Abort, S.L., Cheung, W.K., Kroske-BYstrom, S., Malekzadeh, T. and Janda, J.M. Identification of Aeromonas strains to the genospecies levels n thew clinical laboratory. *Journal of Clinical Microbiology*, 1992; 30: 1262-1266.
2. Adams, M.D., Nickel, G.C., Bajaksouzian, S., Lavender, H., Murthy, A.R., Jacobs M.R and Bonomo, R.A. Resistance to colistin in Acinetobacterbaumanni associated with mutations in the PmrAB two-component system. Antimicrobial Agent Chemotheraphy, 2009; 53: 3628-3634.
3. Akortha, R E. and Egbule, 0.S. Transfer of tetracycline gene (tet^1) Between Replicons in some

enteric bacteria of Diarrhoeal origin from some Hospitals in the South-South Nigeria. *African Journal of Biotechnology*, 2008; 7(18): 3178-3181.
4. Al-Sweih, N.A., Al-Hubail, M.A. Rotimi, V.O. Emergence oftigecyline and colistin resistance in Acenetobacter species isolated from patients in Kuwait hospitals. *Journal of Chemotheraphy*, 2011; 23: 13-16.
5. Al-Tawfiq, J.A. Increasing antibiotic resistance among isolates of Escherichia coli recovered from impatients and outpatients in a Saudi Arabian Hospital. Infections Control and Hospital Epidemiology, 2006; 27: 748-753.
6. Altwegg, M., Steigerwalt, A. G., Alterwegg-Bissing, R., Luthy-Hottensetein, J and Brenner, D.J. Biochemical identification of Aeromonas genospecies isolated from humans. Journal of Clinical Microbiology, 1990; 28: 258-264.
7. Anyanwu MU, Jaja IF, Nwobi OC. Occurrence and characteristics of mobile colistin resistance (mcr) gene-containing isolates from the environment: a review. Int J Environ Res Public Health, 2020; 17: E1028. doi: 10.3390/ijerph17031028.
8. Beno, P., Kremery, and Demitrovicova, A Bacteramia in cancer patients caused by colistin – resistant Gram-negative bacilli after previous exposure to ciproflocin and /or colistin. Journal of clinical microbiology Infection, 2006; 12: 496-500.
9. Bonnin RA, Bernabeu S, Jaureguy F, Naas T, Dortet L. MCR-8 mediated colistin resistance in a carbapenem-resistant Klebsiella pneumoniae isolated from a repatriated patient from Morocco. Int J Antimicrob Agents, 2020; 13: 105920. doi: 10.1016/j.ijantimicag.2020.105920.
10. Borrell, N., Acina, S.G., Figueras, M.J and Martines- Murcia, A j. Identification of Aerornonas clinical isolates by restriction fragment length polymorphism of PCR-amplified 16S rRNA genes. Journal of Clinical Microbiology, 1997; 35: 167: 1—167.
11. Carnahan, A., Behram, S. and Joseph, S.W. Aerokey U: a flexible key for identifying clinical Aeromonas species. Journal of Clinical Microbiology, 1991a; 29: 2843-2849.
12. Carnahan, A., Fanning, G.R. and Joseph, S.W. Aeromonasjandaei (formerly Genospecies DNA Group 9 A. sobria), a new sucrose-ne gative species isolated from clinical specimens. Journal of Clinical Microbiology, 1991; 29: 560—564.
13. Carson, J., Wagner, T., Wilson, T. and Donachie, L. Miniaturized tests for computer-assisted identification of motile Aeromonas species with an improved probability matrix, Journal of Applied Microbiology, 2001; 90: 190—200.
14. Catchpole, CR., Andrews, LM., Breuwald., N. and Wise, R. A reassessment of the in vitro activity of colistinsulphoniethate. Journal of Antimicrobial Ozemotherapy, 1997; 39: 255—260.
15. Collins, M.D., Martiuez-Murcia, Al and Cai, J. Aeromonasenteropelogenes and Aerornonasichüosmia are identical to Aeromonastrota and Aeromonasverond, respectively, by small-subunit rRNA sequence analysis. International Journal of Systematic Bacteriology, 1993; 43: 855—856,
16. Dean, CR., Visalli, MA, Projan, SI, Stun, P.E, Bradford, PA. Efflux mediated resistance to tigecycline (GAR-936) in Pseudomonas aeruginosa PAOI. Antimicrobial Agents Chemotherapy, 2003; 47: 972—78.
17. Egbule, O. S. Ehwarieme, D. A. and Owhe-Ureghe. U. B. High rate of antibiotic resistance in a neonatal intensive care unit of a University Hospital *British Microbiology 'Research Journal*, 2016b; 15(1): 1-6.
18. Egbule, O. S. Owhe-Ureghe, U. B. and Odih E. E. (2016a). Occurrence of multidrug resistance among *E.coli* 0157: H7 isolated from stool samples obtained from Hospitalized children. *Journal of probiotics and Health* 4: 3: 1-4
19. Egbule, O. S. Antimicrobial Resistance and *β*-Lactamase Production among Hospital Dumpsite Isolates. Journal of Environmental protection, 2016; 7(07): 1057-1063.
20. Egbule, O. S. and Yusuf Ibrahim. Multiple antibiotic Resistance in *Escherichia coli* isolated from cattle and poultry faeces in Abraka, south- south Nigeria.*Tropical Agricultural Science*, 2019; 42(2): 585-594.
21. Egbule, OS. Occurrence of extended spectrum beta–lactamases and sul 1 in multi-drug resistant Escherichia coli and Salmonella isolated from poultry feeds. Scientific African, 2022; 18: e01362
22. Egbule, O. S. and Egbule, P. E. Plasmid-borne antimicrobial resistant bacteria isolated from poultry litter. Implication for crop production. *NISEB Journal*, 2016; 16(2): 1595-6938.
23. Esteve, C, Gutierrez, M.C. and Ventosa, A. Aeromonasencheieia sp. isolated from European eels. International Journal of Systematic Bacteriology, 1995; 44: 462-466.
24. Evans, ME, Feola, D.J, and Rapp, R.P. Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram-negative bacteria. Journal of Analytical Pharmacotherapy, 1999; 33: 960-67.
25. Falagas, ME, Bliziotis, LA, Kasiakou, S,K., Samonis, G., Athanassopoulou, P. and Michalopoulos, A. Outcome of infections due to pandrug-resistant Gram-negative bacteria. Journal of Infectious Disease., 2005; 20: 5-24.
26. FaIagas, M.E., Fragoulis, Kit, Kasiakou, S.K., Sermaidis, G.J. and Michalopoulos, A. Nepbrotoxicity of intraivnouscolistin: a prospective evaluation. International Journal of Antimicrobial Agents, 2007; 26: 504-507.
27. Falagas, M.E., Kasiakon, 5K., Kofteridis, D.P., Roditakis, G. and Samonis, G. Effectiveness and nephrotoxicity of intravenous colistin for the treatment of patients with infections due to polymyxin-only-susceptible (POS) Gram-negative

bacteria, European Journal of Clinical Microbiology and Infectious Diseases, 2006; 25: 596499.
28. Falagas, M.E. and Kasiakou. S.K. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Journal of Clinical infectious Diseases, 2006; 1333—1341.
29. Gales, A.C., Jones, RN. and Sader, H.S. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram negative bacilli: report from the SENTRY antimicrobial surveillance programme. Journal of Clinical Microbiology Infection, 2006; 12: 315-321.
30. Gales, A.C., Reis, A.O. and Jones, RN. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. *Journal of Clinical Microbiology*, 2001; 39: 183—190.
31. Graf, J. Diverse restriction fragment length polymorphism patterns of the PCR-amplified 165 rRNA genes in Aeromonasveronii strains and possible misidentification of Aeronionas species. *Journal of Clinical Microbiology*, 1999; 37: 3194—3197.
32. Groisman, E.A., Kayser, I and Soncini, EC. Regulation of polymyxin resistance and adaptation to low environments. *Journal of Bacteriology*, 1997; 179: 7040-7045.
33. H.K. Johansen, S. M. Moskowitz, 0. Ciofu, I. Pressler, and N. Hoiby. Spread of colistin resistant non-mucoid Pseudomonas aeruginosa among chronically infected Danish cystic fibrosis patients. *Journal of Cystic Fibrosis*, 2008; 7: 391-397.
34. Ha nninen, M.L. Phenotypic characteristics of the three hybridization groups of Aeromonashydrophila complex isolated from different sources. Journal of Applied Bacteriology, 1994; 76: 455-462.
35. Ha nninen, M.L. and Siitonen, A. Distribution of Aeromonas phenospecies and genospecies among strains isolated from water, foods or from human clinical samples. *Journal of Epidemiology and Infection*, 1995; 115: 39-50.
36. Helen, W.B., and George H.T. Bad Bugs, No Drugs: No ESCAPE! An Update from the *Infectious Diseases Society of America Journal of Clinical Infectious Diseases*, 2009; 48: 1-12.
37. Hsueh, P.R., Teng, L.J., and Chen, C.Y. Pan drug-resistant Acinetobacter baumannii causing nosocomial infections in a university hospital, Taiwan. Journal of Emerging Infectious Diseases, 2002; 8: 827-832.
38. Huys, G., Coopman, R., Janssen, P. and Kerster, K. High resolution genotypic analysis of the genus Aeromonas by AFLP fingerprinting. International Journal of Systematic Bacteriology, 1996; 46: 572-580.
39. Huys, G., Kampfer, P., Altwegg, M, Coopman, R., Janssen, P., Gillis, M. and Kenters, K. Aeromonaspopoffiisp a mesophilic bacterium isolated from drinking water production plant s and reservoir. International Journal of Systematic Bacteriology, 1997; 47: 1165–1170.
40. Iweriebor, B.C, Egbule, O.S and Obi, L.C. The Emergence of Colistin-and Imipenem-Associated Multidrug Resistance in Isolates from Retail Meat. Polish Journal of Microbiology, 2022; 71(4): 519-528.
41. Jain, R. and Danziger, L.H., Multidrug-resistant acinetobacter infections: an emerging challenge to clinician. Journal of Analytical Pharmacotherapy, 2004; 38: 1449-59.
42. Janda, J.M. and Abbott, S.L. Evolving concept regarding the genus Aeromonas: An expanding panorama of species diseases presentations, and unanswered question. Journal of Clinical Infectious Diseases, 1998; 27: 332-344.
43. Janda, J.M. and Abbott, S.L., Khashe, S. Kellogg G.H. and Shimanda, T. Further studies on biochemical characteristics and serologic properties of the genus Aeromonas. Journal of Clinical Microbiology, 1996; 34: 1930-1933.
44. Karabinis, A. Paramythiotou, E. and Mylona-Petropoulo, D. Colistin for Klebsiellapnemoniaae-associated sepsis Journal of Clinical Infection diseases, 2004; 13: 155-160.
45. Katayama, Y. Ito, T. and Hiramatsu, K. A new class of genetic element, Staphylococcus cassette chromosome mechanism, encodes methicillin resistance in Staphylococcus aureus. Journal of Antimicrobial Agents Chemotherapy, 2000; 44(6): 1549-1555.
46. Khan, R.A. Khan, F. Iqbal, M. and Nigar H. Prevalence and antibiotic Susceptibility of multi-drug resistant Staphylococcus aureus and Acinetobacterbaumanii in clinic sample from intensive care unit patients in tertiary care hospital at Peshawar (Pakistan. Journal of Clinical Microbiology Infections, 2014; 18(3): 231.
47. Kontopidou, F., Planchouras, D. and Papadomicelakis, E. Colonization and infections by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients. Journal of Clinical Microbiology infections, 2011; 17: 9-11.
48. Kuo, L.C., Yu, C.J., Lee, L.N. Clinical features of pan drug-resistent Acinetobacterbaumannii bacteremia at a University Hospital in Taiwan. Journal For Medical Association, 2003; 102: 601-606.
49. Laffineur, K., Janssens, M., Charlier, J., Avesani, V., Wauters, G. and Delme'e, M. Biochemical and susceptibility test useful for identification of nonfermenting gram-negative rods. Journal of Clinical Microbiology, 2002'; 40: 1085-1087.
50. Landman, D. Bratu, S. Alam, M. and Quale, J. Citywide emergence of Pseudomonas aeruginosa strain with reduced susceptibility to polymixin B. Jurnal of Antimicrobia; Chemotheraphy, 2005; 55: 984-957.

51. Lee, J. Patel, G. Huprickar, S., Calfee, D.P. and Jenkin S.G. Decreased susceptibility to polymyxin B during treatment for carbapenem-resistant Klebsiella pneumonia infction. Journal of clinical Microbiology, 2009; 147: 1611-1612.
52. Li J, Nation RL, Milne R.W, Turnidge JD , Coulthard K. Evaluation of colistin as an agent against multi-resistant gram-negative bacteria. *International Journal Antimicrobial Agents*, 2005; 25: 11-25.
53. Magiorakos, A.P. Srinivasan, A. and Carey, R.B. Multidrug-resistant extensively drug-resistant and pan drug-resistant bacteria: an International expert proposal for interim standard definitions for acquired resistance. Journal of clinical Microbiology Infections, 2012; 18: 268-281.
54. Nabti LZ, Sahli F, Ngaiganam EP, Radji N2, Mezaghcha W, Lupande-Mwenebitu D, Baron SA, Rolain JM, Diene SM. Development of real-time PCR assay allowed describing the first clinical Klebsiella pneumoniae isolate harboring plasmid-mediated colistin resistance mcr-8 gene in Algeria. J Glob Antimicrob Resist, 2019; 20: 266-271. doi: 10.1016/j.jgar.2019.08.018.
55. Nordman, P. Naas, T and Poil, L. Global spread of carbapenemas producing Enterobacteriaceae. Journal of Emmerging Infectious Diseases, 2011; 17: 1791-1798.
56. Overman, T.L and Janda, IM. Antimicrobial susceptibility patterns of Aeromonasjandaei, A. schzthenu A. trota and A. veronii biotype vet-anti. Journal of Clinical Microbiology, 1999; 37: 706—708.
57. Pitout, J.D. and Laupland, K.B. Extended-spectrum µ-lactamaseproducingEnterobacteriaceae: an emerging public-health concern. Lancet. Journal of Infectious Diseases, 2008; 83: 159-166.
58. Royer, S. S, Farina, Al,, Miyuki, SI., Chagas, T.P., de Campos, PA, da Fonseca Batistào, D.W., Asensi, MD., GontijoFitho, P2. and Ribas, R.M. Spread of multidrug-resistant Acinetobacterbaumannii and Pseudomnonas aeruginosa clones in patients with ventilator associated pneumonia in an adult intensive care unit at a university hospital. Journal of Infectious Diseases, 2015; 19(4): 350-357.
59. Russotto, V., Cortegiani, A, Graziano, G, Saporito, L, Raineri, S.M., Mammina, C. and Gianatano, A. Bloodstream infections in intensive care unit patients: distribution and antibiotic resistance of bacteria. Journal of Infections. Drug Resistance, 2015; 8: 287-296.
60. Sader, H., David, S., Farrell, J, Flamm, R.K. and Jones, RN. Antimicrobial susceptibility of Grain-negative organisms isolated from patients hospitalized in intensive care units in United States and European hospitals. Journal of Diagnostic Microbiology and Infectious Diseases, 2014; 78: 443448.
61. Saeed, N.K., Kambal, A.M. and E1-Kiiizzi, N.A. Antimicrobial-resistant bacteria in a general intensive care unit in Saudi Arabia. Saudi Medical Journal, 2010; 31(12): 1341-9.
62. Schouten, M.A, Hoogkamp-Korstanje, JA, Meis, IF. and Voss, A. Prevalence of vancomycin resistant enterococci in Europe. *European Journal of Clinical Microbiology and Infectious Diseases*, 2000; 19: 816-22.
63. Sood, S., Malhotra, M, Das, B.K, and Kapil, A. Enterococcal infections and antimicrobial resistance. Indian. *Journal of Medical Microbiology*, 2008; 128(2): 111-121.
64. Souli, M., Galani, I., Antoniadou, A., Papadomichelakis, E., Poulakou, G,, Panagea,T., Vourli, S., Zerva, L., Armaganidis, A., Kanellakopoulou, K. andGiamarellou, H. An outbreak of infection due to beta-lactamaseKiebsiella pneumonia carbapenemase 2-producing K pneumoniae in a Greekuniversity hospital: molecular characterization, epidemiology, and outcomes. *Journal of Clinical Infectious Diseases*, 2010; 50: 364-373.
65. Sueke, H., Marsh, H. and Dhital, A. Using intrathecal colistin for multidrug resistant shunt infection. *Journal of Neurosurgery*, 2005; 19: 51-52.
66. Tan, R., Liu, J., Li, M,, Huang, 1, Sun J. and Qu, H. Epidemiology and antimicrobial resistance among commonly encountered bacteria associated with infections and colonization in intensive care units in a university affiliated hospital in Shanghai. Journal of Microbiology and Immunology Infections, 2014; 47: 87-94.
67. Tsioutia, C., Kritsotakis, E.I, Maraki, S. and Gikas, A. Infections by pan drug-resistant gram-negative bacteria: clinical profile, therapeutic management, and outcome in a series of 21 patients. *European Journal of Clinical Microbiology and infectious Diseases*, 2010; 29: 301-305.
68. Urban, C, Bradford, PA, Tuckmau, M.., Segal-Maurer, S., Wehbeh, W., Grenner, L., Colon-Urban, R. Mariano, N. and Rahal, J.J. Carbapenem-resistant Escherichia coli harboring Klebsiella pneumonia carbapenemase b-lactamasesassociated with long-term care facilities. Journal of Clinical Infectious Diseases, 2008; 46: 127-130.
69. **Wattal,** C, Goel, N. and Oberoi, J. K. Surveillance of Multidrug Resistant Organisms in a Tertiary Care Hospital in Delhi, India. *Journal of Infectious Diseases*, 2010; **53**: 32-36.
70. Wang X, Wang Y, Zhou Y, Wang Z, Wang Y, Zhang S, Shen Z. Emergence of colistin resistance gene mcr-8 and its variant in Raoultellaornithinolytica. Front Microbiol, 2019; 10: 228. doi: 10.3389/fmicb.2019.00228.