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Identification, Characterization and Plasmid Profiling of Multi Drug Resistant Nocomial Pathogens Isolated from Selected Hospitals in Ilorin Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Author ATA designed the study, managed the literature searches, wrote the protocol, and wrote the first draft of the manuscript. Author SEY managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to investigate the distribution and prevalence of some multidrug resistant nosocomial pathogens in various selected hospitals in Ilorin. Various hospitals sections were assessed. This finding revealed that *Klebsiella pneumoniae* was predominant with 14.9% followed by *E. coli*, *Streptococcus* sp and *Acinetobacter* sp of 12.4%, 12.3% and 12.2% respectively. All Gram negative bacteria were susceptible to Ofloxacin, Ciprofloxacin Cephalexin and resistant to Chloramphenicol, Septrin, Vancomycin and Ampicillin while, Gram positive bacteria were found to be susceptible to Ofloxacin, Vancomycin and Ampicillin and resistant to septrin, Augmentin and tetracycline. *Acinetobacter* and Pseudomonas were found to display high level of resistance to most tested antibiotics with varying magnitude. Six of the isolates harboured R-plasmid acquired transfer of mobile genetic element. Gene coding for antibiotics resistance were located on the plasmid while the other three isolates without plasmids may have their gene coding located on their chromosomal DNA. It was concluded that the principle of antimicrobial stewardship is urgently needed to preserve efficacy of available antimicrobial agents.

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1. INTRODUCTION

Nosocomial or hospitalized acquired infections are infections appearing in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission.Such infection manifest within 72hrs or more after admission [1,2,3].

Nosocomial infections are estimated to cause or contribute to nearly 80,000 deaths annually in the United States [4] such infections are serious and public health hazard throughout the world as a matter of fact, is the fourth leading cause of death [5]. These infections remain a major global concern. Overall national prevalence rates have been described as ranging between 3.5 and 9.9% [6,7,8,9].

Hospital-associated infection which has a worldwide distribution remains a major cause of deaths among hospitalized patients [10]. It has been estimated that over 1.4 million people worldwide suffer from infectious complications acquired in the hospital [11,2]. In view of that, David and Famurewa [3] reported that the hospital is no longer a place where sick people recover from their illnesses but also where illnesses at times get complicated and healthy people get infected [1]

Hospital environment play an important role in Nosocomal infection because is a potential reservoir of infectious agents since it houses both patients with diverse pathogenic microorganisms and a large number of susceptible/immune-compromised individuals [12,13]. The nosocomial pathogens that cause infections can come either from endogenous or exogenous sources [14].

The most important means of transmission of nosocomial infections is by contact, usually direct but sometimes indirectly by means of secretions from the body [15].

Hospital surfaces and nursing staff are the most prevalent sources of Nls [7]. Microorganisms are transmitted in hospitals through several routes; nursing staff are the most prevalent sources of the transmission of the bacteria in hospitals [16]. The transfer of micro organisms from environmental surfaces to patients is largely via hand contact with the surface [6,17,7]

In a report of Bolaji et al. [18] who explain that the greatest threat to the use of antibiotics is the emergency and spread of resistance in pathogenic bacteria that consequently cannot be treated by previously successful regimens [2]. Available therapeutic options for antibiotic resistant organisms are severely limited, as these organisms frequently display a multidrug resistant (MDR) phenotype [19,20,14,21,22]. There is no doubt that the use of antibiotics provides selective pressure that result in antibiotic resistant bacteria and resistance genes. While some resistance bacteria are found naturally in the environment, pathogens and non-pathogens are released into the environment in several ways, contributing to a web of resistance that includes humans, animals, and the environment, essentially the biosphere [18].

The spread of mobile genetic elements such as plasmids, transposons, and integrons has greatly contributed to the rapid dissemination of antimicrobial resistance among several bacterial genera of human and veterinary importance [23]. Antimicrobial resistance genes have been shown to accumulate on mobile elements, leading to a situation where multidrug resistance phenotypes can be transferred to a susceptible recipient via a single genetic event [24].

The spread of antibiotic resistance genes may be causally related to the overuse of antibiotics in human health care and in animal feeds, increased use of invasive devices and procedures, a greater number of susceptible hosts, and lapses in infection control practices leading to increased transmission of resistant organisms. The resistance gene sequences are integrated by recombination into several classes of naturally occurring gene expression cassettes and disseminated within the microbial population by horizontal gene transfer mechanisms: transformation, conjugation or transduction [25,24].

The increasing prevalence of antimicrobial resistant bacterial pathogens has severe implications for the future treatment and prevention of infectious diseases in both animals and humans. The versatility with which bacteria adapt to their environment and exchange DNA between different genera highlights the need to implement effective antimicrobial stewardship and infection control programs in view of this investigation on the occurrence and distribution of

multidrug resistant nosocomial pathogens in selected private and government hospitals in Ilorin was carried out.

2. MATERIALS AND METHODS

Twenty five hospitals within Ilorin metropolis were investigated. Hospital personnel, hospitalized patients, environmental surfaces, various sections such as Wards, Pharmacy sections, record section, waiting rooms, Nurses palms & Gown and Patient palms & Bed sheets were all investigated.

Sterile swab sticks moistured in nutrients broth were used for taking samples from hand contact surfaces and palms. A sedimentation technique was used for the isolation of airborne bacterial contamination by exposing nutrient agar plates both in the general toilets and various sections for 10mins. The samples were taken to the laboratory and analyzed immediately. The swabs were streaked on Nutrient agar (NA, Oxoid), MacConkey agar, Blood agar, Eosin Methylene Blue (EMB) agar and Manitol Salt Agar.

2.1 Culture and Identification

All the colonies developed after 24hours of incubation were subcultured several times to obtain stock culture. Preliminary identification of bacteria was based on colonial morphology of the organisms. Such as haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Biochemical tests were performed on colonies from primary cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests (oxoid). Namely: Kliger Iron Agar (KIA), Indole, Simon's citrate agar, Lysine Iron Agar (LIA), urea and motility. Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test results.

The criterion for an isolate to be multi drug resistant (MDR) was defined as being resistant to three or more drugs of different structural classes [26] Only isolates that showed multidrug resistance were selected for this study.

2.3 Antibiotic Susceptibility

The antibiotics susceptibility patterns of the isolates to common antibiotics were determined using method of Bauer et al. [27] using Mueller-Hinton Agar.

Five discrete colonies of different isolated colonies were inoculated into 5ml of trypticase soy broths and incubated at 35ºC for 5hours the culture turbidity was matched with 0.5 Macfarland standards and spread over the Mueller-Hinton agar (oxoid) using sterile cotton swab and allowed to dry. The appropriate antibiotics multi-discs were placed in the agar aseptically using sterile forcep the plates were then incubated at 37ºC for 24hours. The commercial antibiotics discs used in this study were Ofloxacin, 30µg; Ciprofloxacin, 5µg; cefrixone, 30µg; ceftazidime, 30µg, amoxicillin, 25µg, augmentin, 30µg , gentamicin, 10µg; tetracycline 10µg, ampicilin, 30µg, streptomycin, 30µg; erythromycin,10µg; vancomycin, 10µg;septrin 10µg; rifampicin,10µg (Oxoid). The interpretation was done using zone of inhibition sizes. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler [28,21,29]. All the results were taken in triplicate and the standard error was calculated for each parameters.

2.4 Plasmid Profiling and Agar Gel Electrophoresis

Plasmid profiling of the isolates that were resistant to up to five antibiotics were carried out. Plasmid extraction was carried out using the method described by Ojo and Oso [30] with slight modification. Pure isolates were inoculated on tars broth and incubated. The grown cells were harvested and suspended in 200 µL of solution A [100 mm glucose-50 mm tris hydrochloride (pH 8)-10 mm ethylenediaminetetraacetic acid (EDTA)] containing 10mg tanolysin and incubated for 30 min at 37ºC in a dodecyl sulphate in 0.2 µL NaOH was added and the samples were mixed by inverting tubes. Three hundred uL of a 30% potassium acetate solution (pH 4.8) was added and the samples were mixed by vortexing. The supernatant was removed and extracted once with a phenol – chloroform mixture (1:1) and precipitated with an equal volume of isopropanol. The plasmid DNA was then dissolved in 100 µL of tris-EDTA buffer. Electrophoresis of the DNA was carried out on a 0.8% agarose gel in a 0.5×concentration of tris-borate-EDTA buffer. After boiling, the solution was allowed to cool 10 µL of ethidium bromide was added to the cooled agarose solution. This was poured into a casting tray with a comb placed across its rim to form wells. The gel was allowed to set for 30 min and the comb was removed. Twenty µL of the plasmid DNA

samples were then loaded into the wells after mixing with 2 µL of bromophenol blue.

The gels were thereafter electrophoresed in a horizontal tank at a constant voltage of 60v for about 1 h and 30 min. After electrophoresis, plasmid DNA bands were viewed by fluorescence of bound ethidium bromide under a short wave ultraviolet light transilluminator and the photograph were taken using a digital camera.

3. RESULTS AND DISCUSSION

The results of this present finding demonstrated that both private and government hospitals in Ilorin harbored pathogenic bacteria. Table 1 shows the distribution and frequency of the nosocomial pathogens in the hospitals. Plethora of bacterial isolates displayed high level of multidrug resistance. *Klebsiella* spp were found to be predominant with 14.9%, mostly in the wards and pharmacy sections, followed by *E. coli* (12.4%) frequent in pharmacy section and wards, *Pseudomonas sp* (6.6%) which was majorly found in the toilet and the wards appeared to be the least among all the isolates while *Streptococcus* sp., *Bacillus* sp, and *Staphylococcus* sp were found to be 12.3%, 11.5% and 11.3% respectively. While, *Enterococcus* sp (9.1%), *Bacillus* sp and *Streptococcus* sp. were prevalent on the patients' palm and bed sheets while *Staphylococcus* sp was predominant both in the patients and the nurses' palms. The *Proteus* sp was found to be 9.5% on the whole, it was found to be most prevalent bacteria within the wards sections both in the air, walls and floor followed by *Klebsiella pneumonia*, *E. coli*, *Streptococcus* sp and *Acinetobacter* sp. While *Enterococcus* sp found to be the least. All above mentioned pathogens have been described to cause different nosocomial infections [19,20,31,14,22]. *Streptococcus* sp and *Bacillus* sp were mostly prevalent on the patient palm and on formites (bed sheet), this is in line with Presott et al. [32] who reported that the formites are involved in the transmission of pathogens in health care environments. *Klebsiella pneumoniae* was found to be the highest in waiting room and pharmacy section. *Acinetobacter* sp found to be mostly occurred in the hospital toilet.

Jalalpour and Ebadi [33] isolated *Stapylococcus* spp, *Bacillus* spp and Enterobacteriaceae from nursing staff this is in agreement with present finding. Also reported *Staphylococcus aureus* and *Pseudomonas* to be most dominant organisms isolated from the delivery rooms at all examined hospitals. Jalalpoor [34] reported high frequency of antibiotics resistant strains on hospital surfaces.

The most commonly isolated bacteria were *Proteus sp, Klebsiella pneumonia* and *E. coli* all from the various sites within the ward. Also *Klebsiella pneumonia* and *Acinetobacter* sp were found both in the pharmacy section and Toilet in large proportion. *Klebsiella pneumonia* was prevalent in nurses' gown, serving trays and palms followed by *Staphylococcus* sp and *Enterococcus* sp. Udeze et al. [35] isolated similar bacteria from various hospital sites and sections. Mohiuddin et al. [36] described high level of resistance of the isolated pathogens to commonly used antibiotics.

The present investigation demonstrated that hospital personnels, patients, various environmental surfaces and formites habour multidrug resistant nosocomial pathogens. Mohiudin et al. [36] reported that predominating organisms responsible for nosocomial infection in their findings were *E. coli* (55.9%), *Pseudomonas sp* (33.3%), *Proteus* sp (12.7%), *Staphylococcus aureus* (5.9%), *Klebsiella sp* (4.9%) and *Acinetobacter sp* (3.9%).

This present finding agreed with the results of Tambekar et al. [37] who isolated *Pseudomonas aeruginosa* from indoor air of hospitals and Ekrami et al. [38] also reported that the Presence of bacteria was different from ward to ward hospital to hospital based on activities of each hospital.

Table 2 shows antibiogram pattern of the isolates. The isolates were resistant to one or more drug of different structural classes. All the Gram negative bacteria were found to be susceptible to ofloxacin, ciprofloxacin, cephalexin, pefloxacin, while they were resistant to septrin and vancomycin .Antibiotic susceptibility tests revealed that all the isolates were susceptible to Ofloxacin, Ciprofloxacine and Cephalexin this is in agreement with Okonkwo et al. [21]. Eighty percent of However, all Gram negative bacteria were resistant to vancomycin in agreement with the work of Nkang et al. [22]. *Acinetobacter* sp, *Staphylococcus* sp and *Pseudomonas* sp observed to show varying degree of resistance to augmentin, chloramphenicol and amoxicillin. Meanwhile, *Proteus* sp and *Klebsiella sp* were susceptible to augmentin while streptomycin showed inhibitory

effect on *Proteus sp* and *E. coli* .It is also worthy to note that the wide ranged resistances showed by some of the bacteria strains especially the *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus* sp and *Bacillus* sp against augmentin , ampicillin may be due to the activity of beta-lactamase enzymes [39].

Proteus sp, E. coli, Klebsiella sp and *Pseudomonas* sp were susceptible to gentamycin but resistant to amoxicillin. *Proteus* sp*, Acinetobacter* sp, *Klebsiella sp, Bacillus* sp*, Streptococcus* sp and *Pseudomonas* sp were resistant to tetracycline but *E. coli* was susceptible to *Acinetobacter* sp and *Pseudomonas* sp were found to be resistant to high numbers of tested antibiotics. They were both resistant to erythromycin and augmentin, only *Acinetobacter* sp found to be resistant to gentamycin and also *Pseudomonas* sp show resistance to rifampicin.

Bacillus sp, Enterococcus sp, Streptococcus sp, Acinetobacter sp, Klebsiella sp and *Pseudomonas* sp were found to be resistant to streptomycin. *Staphylococcus sp, Proteus sp, E.coli, Klebsiella* sp showed wide range of resistance to amoxicillin, meanwhile, all other remaining tested Gram positive isolates were susceptible to amoxicillin.

Only *Enterococcus* sp displayed resistance to pefloxacin among all the Gram positive isolates. *Bacillus* sp, Streptococcus sp and Staphylococcus sp were resistant to augmentin, septrin and rifampicin. *Bacillus sp, Enterococcus* sp and all Gram negative were resistant to vancomycin .

The antibiotics sensitivity test of this work shows that ofloxacin, ciprofloxacin and cephalexin were mostly effective against all the pathogens. This agreed with the finding of Okonko et al. [21].

Both *Pseudomonas* sp and *Acinetobacter* sp were both resistant to Erythromycin, augmentin and chloramphenicol. These antibiogram patterns are in concordance with previous findings of Okonko et al. [21] who reported 100% resistance to septrin by Gram negative isolates but deviated by 50% resistance by gram positive isolates.

Mohiuding et al. [36] also reported that resistant rate of *Staphylococcus aureus was* relatively lower than that of Gram negative bacteria and this can be attributed to the production of extended spectrum beta-lactamase by Gram negative organism [40]. However, mechanism of antibiotics resistance in these gram-negative bacteria could be attributed to loss of porin, production of β-lactemasses and increase expression of efflux pumps. Environmental changes might also select antibitics resistant bacteria or antibiotics resistance genes without the need of antibiotic selective pressure [41] MDR pump can efflux, not only antibiotics, but other toxic compounds like solvents [42], detergents, Ma et al. [43] or biocide, Sanchez et al. [44] use for cleaning and disinfection. Although it has been demonstrated, enrichment in bacteria capable to resist these agents and thus antibiotic resistant might be possible in contaminated environments [45].

Hota [46] reported that the major reservoirs for MRSA include colonized or infected patients, personnel in the hospitals and major mechanism is done via the unwashed hands of health care workers. Ogbolu et al. [47] demonstrated high level of multi drug resistance among gram negative enteric bacteria from patient in Nigeria hospitals, containing transferable β-lactamase and quinolone resistance alleles.

Acinetobacter baumannii isolated has not been reported to be found in any Nigerian hospitals however, drastic measure is needed to put in place because some of these isolated pathogens, such as MDR *Klebsiella pneumonia* and *Acinetobacter baumannii*, reported to be virtually untreatable with current antibiotics [48]. This is also supported with the report of Maragakis and Parl [49] who said that infection caused by multidrug resistant *A. baumannii* is currently among the most difficult one to treat. Kyungiwin Lee et al. [50] also emphasized that *Acinetobacter baumannii* causes various nosocomial infections with high mortality; therefore, occurrence of *Acinetobacter* sp in our health care centre is worrisome.

Cornaglia et al. [51] reported that *Enterobacter cloacae* is one of the bacteria which is needed to place on surveillance in health care centre because their implication in any infection is largely associated with decreased immunity Nkang et al. [22] attributed high percentage of the resistance to the antibiotics to their prevailing usage and abuse in the area under study.

Table 1. Frequency and Types of Major Bacterial Isolates

Samples	*Klebsiella pneumonia*	*Acinetobacter sp*	*Proteus sp*	*E. coli*	*Bacillus sp*	*Enterococcus sp*	*Streptococcus sp*	*Staphyloccus aureus*	*Pseudomonas sp*
Wards (Air, Walls & Floor)	36	26	42	30	22	14	31	22	13
Patients Palm & Bed Sheets	28	20	08	12	33	11	36	16	21
Record Sections (Air &Tables)	20	21	10	18	23	08	26	17	10
Waiting Room (Air, Walls & Chairs)	25	16	10	17	13	07	22	14	18
Pharmacy Section (Air, Tables & Walls)	32	20	16	31	22	24	18	18	11
Toilet (Air & Walls)	15	31	21	26	18	26	11	22	08
Nurses (Gown, drug serving trays & Palms)	27	16	09	18	11	22	07	29	-
Percentage of Occurence	14.9%	12.2%	9.5%	12.4%	11.5%	9.1%	12.3%	11.3%	6.6%

Table 2. Susceptibility Pattern of the Isolates

ISOLATES	OF	E	CPX	GN	CX	AMP	AU	CH	AM	PEF	S	SXT	TETRA	VA	RF
Bacillus sp	28.32+0.63	24.81±1.21	26.10±2.81	22.00±2.15	32.65±2.19	7.00±0.12	2.00±0.01	5.68±0.11	21.10±2.08	31.20±3.01	-	-	-	-	10.00±1.20
Proteus mirabilis	20.40±0.99	23.00±2.61	31.20±2.91	24.10±1.09	34.00±3.01	30.00±2.92	29.00±2.51	15.10±0.99	7.00±0.17	22.00±1.05	20.10±2.10	5.00±0.06	15.00±1.23	3.50±2.53	26.00±2.15
Acinetobacter sp	35.60±2.80	14.20±0.68	28.80±2.08	5.00±0.12	26.10±2.32	10.40±1.68	6.00±0.16	8.50±0.19	11.10±1.53	30.10±3.11	14.50±0.69	13.40±0.87	7.00±0.81	8.00±0.90	24.10±2.53
E. coli	36.00±3.11	5.00±0.09	31.10±2.08	36.10±2.92	40.10±3.15	24.20±2.08	8.00±0.15	35.00±2.15	3.00±3.04	25.00±.2.01	20.40±1.29	12.00±0.89	23.00±2.16	6.00±0.68	33.00±2.53
Klebsiella sp	30.00±2.98	36.00±3.02	25.00±2.92	42..00±3.15	40.00±3.02	8.00±0.13	21.00±2.03	29.00±2.19	12.00±1.69	20.00±2.08	6.00±0.06	10.00±0.16	5.00±0.85	2.00±2.12	36±3.15
Enterococcus sp	36±3.08	13.00±1.02	28.00±3.12	40.00±3.86	30.00±2.15	-	36±2.92	9.00±0.12	18.00±1.01	5.00±0.98	13.00±1.01	26.00±2.85	32.00±3.11	10.00±0.54	18±0.99
Streptococcus sp	27.00±2.88	14.00±1.12	28.00±3.12	10.40±0.83	30.00±2.57	24.00±2.53	15.00±0.96	11.00±1.06	21.00±1.93	26.00±1.23	10.00±0.68	16.00±1.62	7.00±0.61	23.00±2.81	31±2.85
Staphylococcus sp	33±2.90	26.002.11	36.00±2.81	41.00±3.14	38.00±3.01	30.00±3.01	12.00±1.01	10.00±0.65	7.00±0.08	24.00±2.01	21.00±2.00	13.00±0.98	18.00±1.23	22.00±2.81	15±1.02
Pseudomonas sp	25.00±2.50	08.00±0.12	18.00±1.32	26.00±2.14	27.00±2.39	25±2.06	10.00±0.34	8.00±0.13	5.00±0.08	23.00±2.81	3.00±0.12	16.00±0.96	16.00±0.95	4.00±0.09	14.00±0.23

OF-Ofloxacin, E-Erythromycin, CPX-Ciprofloxacin, GN-Gentamicin, CX-Cephalexin, AMP-Ampicillin, AU-Augmentin, CH-Chloramphenicol, AM-Amoxillin, PEF-Pefloxacin, S-Streptomicin, SXT-Septrin, Tetra- Tetracyclin, VA-Vancomycin, RF-Rifampicin

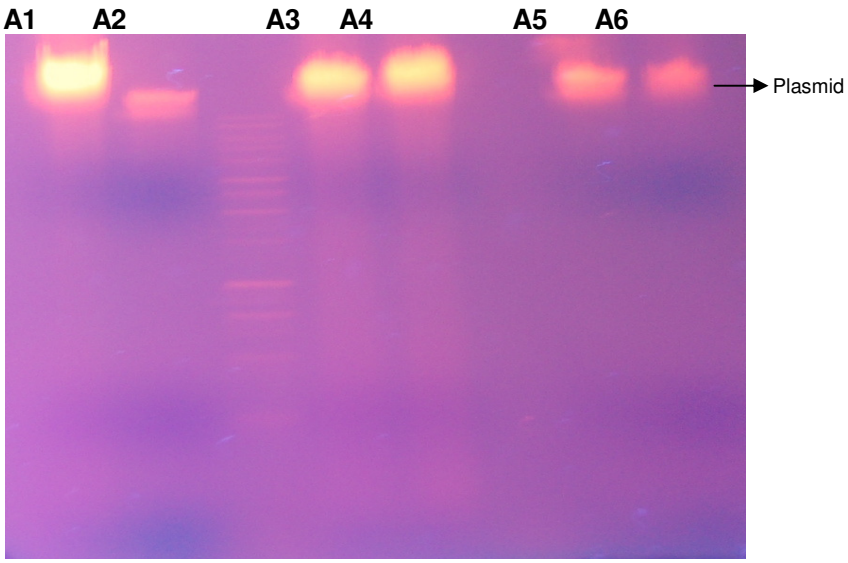

Fig. 1. Plasmid profiles of multidrug resistant isolates. Lane A1=*Acinetobacter* sp; Lane A2=*Bacillus* sp.; Lane A3=*Pseudomonas* sp.; Lane A4=*Staphylococcus* sp.; Lane A5=*E. coli*; Lane A6=*Klebsiella pneumoniae*

Six bacterial isolates *Bacillus* sp, *Acinetobacter* sp., *E. coli, Klebsiella pneumonia*, *Staphylococcus* sp and *Pseudomonas* sp. harboured plasmid while the remaining three of the isolates seemed not to posses plasmids in this present finding shown in Fig. 1. Plasmid curing was achieved only by growing the strain treatment with 10 % sodium dodecyl sulphate (SDS).

Plasmid curing was carried to ascertain the position of R-resistance. It was confirmed that the R-factor was located on plasmid because all the isolates earlier resistant to the tested antibiotics became susceptible after curing. *Proteus* sp., *Enterococcus* sp. and *Streptococcus* sp did not posses plasmid still showed wide range of resistance to various antibiotics. The only hypothesis that can explain such phenomenon is either the resistance gene in the three non-plasmid isolates is present in the chromosome, transposon or integron associated with chromosome rather than plasmid as such both cases are worrisome if they are located on mobile genetic elements if other isolates can acquire their r gene through horizontal gene transfer.

This is in agreement with the work of Oleghe et al. [39] who isolated resistance plasmids (R-factor) from *Bacillus anthracis* and *Staphylococcus aureus*. The gene coding for antibiotics resistance may either coded on the plasmid and chromosomal DNA. Van Hal et al. [52] suggested that the MDR plasmids could be acquired by susceptible bacteria during treatment with antibiotics that can induce and select for horizontal transfer.

However, Bacterial antibiotics resistance patterns associated with the presence of large plasmids and the ability of plasmids for conjugation process. Plasmids which can be transconjugated usually posses a high molecular weight [53].It may be plausible to suggest that the possession of plasmids may have acquired their resistance through selective pressure from increased use and misuse of antimicrobial agents.

Oleghe et al. [39] presumed that the acquisition of resistance may be due to chromosomal mutations or through plasmids that are capable of transfer from one strain of organism to another even across the species in addition to environmental influence. Gram negative bacilli harbour series of antibiotic resistant genes which can be transferred to other bacteria horizontally [54,31]. Plasmids mediated resistance to various antimicrobial drugs have been demonstrated by various workers [55,56,57].

4. CONCLUSION

Since it is evident and established that some of the isolates from hospitals in Ilorin, could pose a

serious health hazard. Their occurrence and prevalence could be attributed to poor hand hygiene, cross contamination between the patients and hospital personnel, misuse of drug can lead to multidrug resistance which may lead limited choice of antimicrobial agents and transfer of such MDR to un-pathogenic enteric organisms. aplication of strict prevention strategies, including changes in antibiotic treatment regimens, hygiene measures, infection prevention and control of horizontal nosocomial transmission of organisms is desirable.On the whole, principle of antimicrobial stewardship is urgently needed to preserve efficacy of available antimicrobial agents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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