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Nosocomial *Acinetobacter* Infections in Intensive Care Unit

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ABSTRACT

Acinetobacter plays an important role in the infection of patients admitted to hospitals. *Acinetobacter* are free living gram-negative coccobacilli that emerge as significant nosocomial pathogens in the hospital setting and are responsible for intermittent outbreaks in the Intensive Care Unit. The aim of this study was to determine the prevalence of *Acinetobacter* in patients admitted into the Intensive Care Unit and determine their role in infections in the ICU. A total of one hundred patients were recruited for the study, catheter specimen urine, tracheal aspirate and blood culture were collected aseptically from the patients. The specimens were cultured on blood and MacConkey and the organisms identified using Microbact 12E (0xoid). The Plasmid analysis was done using the TENS miniprep method. Fourteen (14%) of the 100 patients recruited into the study, developed *Acinetobacter* infection. *Acinetobacter* spp constituted 9% of the total number of isolates. Twelve (86%) of the isolates were recovered from tracheal aspirate, 1(7%) from urine and 1(7%) from blood. All of the isolates harbor plasmids of varying molecular sizes. Ten of the fourteen *Acinetobacter* were isolated at about the same period of time in the ICU with 6(42.7%) having plasmid size in the 23.1kb band and all showed similar pattern revealing that the isolates exhibit some relatedness. The clonal nature of the isolates suggest that strict infection control practices must be adopted in ICU, also an antibiotic policy must be developed for the ICU to prevent abuse of antibiotics that may lead to selection of resistant bacteria.

Keywords: *Acinetobacter*, Intensive Care Unit

1. INTRODUCTION

The control of hospital-acquired infection caused by multidrug resistant Gram-negative bacilli has proved to be a peculiar problem. In the 1970s an increase in the resistant members of the family *Enterobacteriaecea* involved in nosocomial infections followed the introduction of newer broad spectrum antibiotics in hospitals and this led to an increase in the importance of aerobic Gram-negative bacilli, including *Pseudomonas aeruginosa* and *Acinetobacter* spp (Gerner-Smidt, 1994).

Acinetobacter is ubiquitous, free-living and fairly stable in the environment (Joshi *et al*., 2006). Due to their distinct adhesive ability to epithelial cells; they have a predilection to colonize skin, especially in the areas of the perineum, inguinal region, axillae, mucous membranes and upper respiratory airway and cause human infections which include pneumonia, septicemia, wound sepsis, urinary tract infection, endocarditis and meningitis (Joshi *et al*., 2006; Garcia-Garmendia *et al*., 2001a).

Acinetobacter infections are increasingly implicated in infections in intensive care units and have been cited in up to 17% of Ventilator Associated Pneumonia, second only to *Pseudomonas*, which was responsible for 19% of Ventilator Associated Pneumonia in an ICU (Munoz-Price and Weinstein, 2008). In a review from the CDC, 7% of ICU-acquired pneumonias were due to

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Acinetobacter in 2003 compared to 4% in 1986 (Gaynes *et al*., 2005).

Infections caused by *Acinetobacter* are difficult to control due to multi-drug resistance, this limits therapeutic options in critically ill and debilitated patients especially from intensive care units where their prevalence is most noted (Joshi *et al*., 2006).

Acinetobacter outbreaks have been traced to contamination of respiratory-therapy and ventilator equipment from cross-infection by the hands of health care workers who have cared for colonized, infected patients or touched contaminated fomites (Villegas and Hartstein, 2003; Maragakis *et al*., 2004).

A. baumannii, A. calcoaceticus and *A. lwoffii* are the *Acinetobacter* species most frequently reported in clinical literature (Garcia-Garmendia *et al*., 2001b). There is some data to suggest that the proportion of Intensive Care Unit (ICU)-acquired pneumonia cases being found to be due to *A. baumannii* is on the increase.

In large surveillance studies from the United States between 5 and 10% of cases of ICU-acquired pneumonia were due to *A. baumannii*. Data on *Acinetobacter* in Africa is largely limited to South Africa at the present time, although there are scattered reports from other countries in Africa (Lowman *et al*., 2008).

Prior to this report there has been no published data on *Acinetobacter* infection in the ICU of University College hospital Ibadan. *Acinetobacter* infection in literature is associated with high morbidity, mortality and increased length of hospital stay especially amongst patients in ICU, this has been attributed to its ability to acquire and up regulate resistance genes (Garcia-Garmendia *et al*., 2001a).

The aim of the study was to determine the prevalence of *Acinetobacter* in the intensive care unit and also to determine the relatedness of the isolates

2. MATERIALS AND METHODS

This cross sectional study was carried out in the University College `Hospital (UCH) Ibadan, Nigeria. A total of one hundred patients were recruited into study from the ICU which is12 bedded and has a monthly turnover of 25 patients. This population of patients comprised of patients who have had surgery and are on ventilators or intubation with a prior history of antibiotic use and those who have had any form of instrumentation.

Ethical approval was obtained from the University of Ibadan/University College Hospital joint Ethical Committee.

A written informed consent was also obtained from guardians, spouse, parent or caregiver of each participant, thereafter relevant medical history, socio-demograhic data and other information obtained from the care giver and case files were entered into a study proforma.

2.1. Specimen Collection and Transport

Tracheal aspirate, blood and catheter specimen urine were collected from all recruited patients for microscopy, culture and sensitivity. Specimens were collected using aseptic technique to prevent contamination.

For optimal results, specimens were collected in clean sterile, wide bore containers. The samples were collected in patients who had spent at least 48 h in ICU.

2.2. Microscopy

A Gram stain was done on smears made from specimens and then viewed under the light microscope at x100. Classically *Acinetobacter* spp. appear as; short, plump, Gram-negative rods.

2.3. Culture and Identification

The specimen was inoculated on MacConkey agar and blood agar and incubated at 35-37°C for 18-24 h. *Acinetobacter* species grew on MacConkey agar appearing as non lactose fermenters.

All Gram-negative coccobacilli isolated were tested for catalase and motility. All catalase positive, non-motile Gram negative coccobacilli were subjected to an oxidase test. All oxidase negative organisms were inoculated into peptone broth for about 30 min. Subsequently 1ml of the broth was inoculated into the various cups of Microbact Identification kit (Oxoid) and incubated for 18-24 h. After the stated period, Gram negative coccobacilli were identified as *Acinetobacter* spp based on the reactions on the identification panel which was read with the help of the identification software that accompanied the kit.

2.4. Plasmid Analysis

The plasmid analysis was done in collaboration with the molecular biology laboratory of the Nigerian Institute of Medical Research Yaba Lagos, Nigeria, using the

TENS method. This was done to determine the relatedness of the isolates.

2.5. Plasmid Extraction

About 1.5 mL of overnight culture was centrifuged at 10,000 rpm for 1 min in a micro-centrifuge to pellet cells. The supernatant was gently decanted leaving 100 µL together with cell pellet; the resulting suspension was vortexed at high speed to re suspend cells completely.

A volume of 300 µL of TENS was added to the mixture and mixed until the mixture becomes sticky, 150 µL of 3.0M sodium acetate pH 5.2 was added to the mixture and vortexed to mix completely.

The mixture was spun for 5 min in a micro-centrifuge to pellet chromosomal DNA and supernatant was discarded, the pellet was rinsed twice with 1 mL of 70% ethanol and the pellet re-suspended in 40µl of distilled water for further use:

(TENS composition: Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5%.)

2.6. Agarose Gel Electrophoresis

Agarose Gel Electrophoresis was the separation method used to separate DNA based on their molecular weight. A load of 0.8 g of agarose powder was added to 100 mls of TAE buffer and dissolved by boiling. It was allowed to cool to about 60°C then 10 uL of ethidium bromide added and mixed by swirling gently. The agar was poured into electrophoresis tray with the comb in place to obtain a gel thickness of about 4-5mm. It was allowed to solidify then comb removed and the tray placed in the electrophoresis tank.

TAE buffer was poured into the tank ensuring that the buffer was covering the surface of the gel. A volume of 10 µL of sample was mixed with 2 µL of the loading dye and the samples were carefully loaded into the wells created by the combs. The marker was loaded on lane 1 followed by the samples. The electrodes were connected to the power pack in such a way that the negative terminal was at the end where the sample has been loaded. Electrophoresis was run at 60 volt until the loading dye had migrated about three-quarter of the gel. The electrodes were disconnected and turned off and the gel viewed on a UV-transilluminator.

TAE: (Tris, Acetic acid and EDTA).

2.7. Data Analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 15.0. Data were presented using frequency tables, charts, as appropriate and cross tabulation to study relationships and association between variables.

3. RESULTS

A total of one hundred patients were recruited into the study over a period of nine months. The age of the patients ranged from 2 years to 95 years. Majority of the patients (40%) were in the 31-40year age group while the 10-20 year age group constituted the least age group (4%). There were 52 males and 48 males, giving a male to female ratio of 1.08:1. Forty Eight (48%) were Christians, forty seven (47%) were Muslims and 5% were traditionalists. Eighty one (81%) of the patients were admitted from the Accident and Emergency unit while 19% were from other wards in the hospital, Fifty eight (58%) of the patients were resident in Ibadan while forty two (42%) of the patients were from outside Ibadan (**Table 1**).

Table 1. Sociodemographic data of ICU patients

Variable	Number	Percentage
Age (years)		
<10	13	13.0
11-20	4	4.0
21-30	13	13.0
31-40	30	30.0
41-50	20	20.0
51-60	10	10.0
>60	10	10.0
Sex		
Male	52	52.0
Female	48	48.0
Source of admission		
A/E	82	82.0
Ward	18	18.0
Residence		
Ibadan	58	58.0
Outside Ibadan	42	42.0
Religion		
Christian	48	48.0
Islam	47	47.0
Traditionalists	5	5.0

Table 2. Frequency distribution of isolates from patient samples

Organisms	Frequency	Percentage
Tracheal Aspirate		
Acinetobacter spp	12	12
Candida albicans	1	1
Citrobacter freundii	1	1
Enterobacter spp	1	1
Escherichia coli	9	9
Escherichia vulneris	1	1
Klebsiella pneumonia	10	10
Klebsiella aerogenes	7	7
Klebsiella oxytoca	3	3
Proteus mirabilis	3	3
Proteus vulgaris	2	2
Pseudomonas aeruginosa	10	10
Staphylococcus aureus	11	11
Viridans Streptococci	3	3
Urine		
Acinetobacter spp	1	1
Candida albicans	4	4
Citrobacter freundii	1	1
Enterococcus faecalis	1	1
Escherichia coli	8	8
Klebsiella ozaenae	2	2
Klebsiella aerogenes	1	1
Klebiella oxytoca	6	6
Klebsiella pneumonia	9	9
Proteus mirabilis	1	1
Proteus vulgaris	3	3
Pseudomonas aeruginosa	2	2
Staphylococcus aureus	6	6
Blood		
Acinetobacter spp	1	1
Candida albicans	1	1
Citrobacter freudii	1	1
Escherichia coli	3	3
Klebsiella aerogenes	3	3
Klebsiella oxytoca	1	1
Klebsiella ozaenae	3	3
Pseudomonas aeruginosa	1	1
Staphylococcus aureus	17	17

Acinetobacter spp. was isolated from fourteen (14%) of the total number of patients recruited into the study and was responsible for 14% of infections in the ICU based on evaluation of clinical charts. It represented 9% of the isolates from all the specimens collected during the study period (**Table 2**). *Acinetobacter* was responsible for 1% of urinary tract infections, 14% of respiratory tract infections and 1% of blood stream infections.

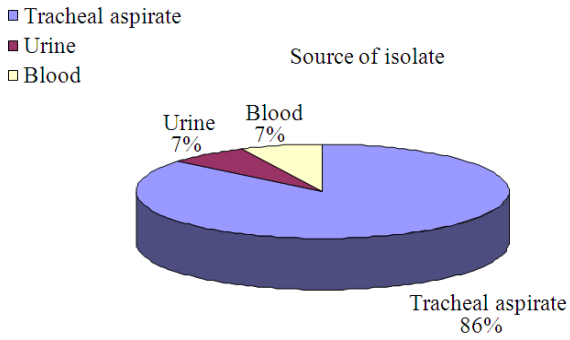

Fig. 1. Frequency distribution of isolates

Twelve (86%) of the isolates were recovered from tracheal specimens while 1(7%) was from urine and blood specimens each. Eleven (79%) of the isolates were *Acinetobacter baumannii* while the others were *Acinetobacter iwoffi* (14%) and *Acinetobacter calcoaceticus* (7%), (**Fig. 1**).

All the isolates in the study harbor plasmids of different molecular sizes. Ten of the fourteen isolates were isolated at about the same period in the ICU with 6(42.7%) having plasmid size in the 23.1kb band and having similar pattern showing that they may be related. The remaining 9(67.3%) did not show any appreciable bands that were related to the others (**Fig. 2**).

4. DISCUSSION

The prevalence of *Acinetobacter* infection in this study was 14%, this high rate of *Acinetobacter* infection may be attributed to the poor infection control practices in the ICU of the Hospital. The observed prevalence is higher than reports from similar studies carried out in France by Joly-Guillou (2005) who reported 9% and Iregbu *et al*. (2002) who reported 4.6% in Lagos, Nigeria (Iregbu *et al*., 2002). *Acinetobacter* constituted 9% of all isolates in the study, this finding is low compared to 14.5% obtained by Kessaris *et al*. (2006), 13.9% by Raka *et al*. (2004) but higher than 8.4% reported by Oberoi *et al*. (2009) and 3% reported by Iregbu *et al*. (2002). This may be because their study included all patients in the hospital compared to this study which was limited to the ICU.

The incidence of *Acinetobacter* blood stream infection in this study was 1.3%, this is a little lower than 2% reported by Michalopoulos and Falagas (2007) and 8.8% reported by Garcia-Garmendia *et al*. (2001b) This difference may be due to the lower number of patients recruited in the study.

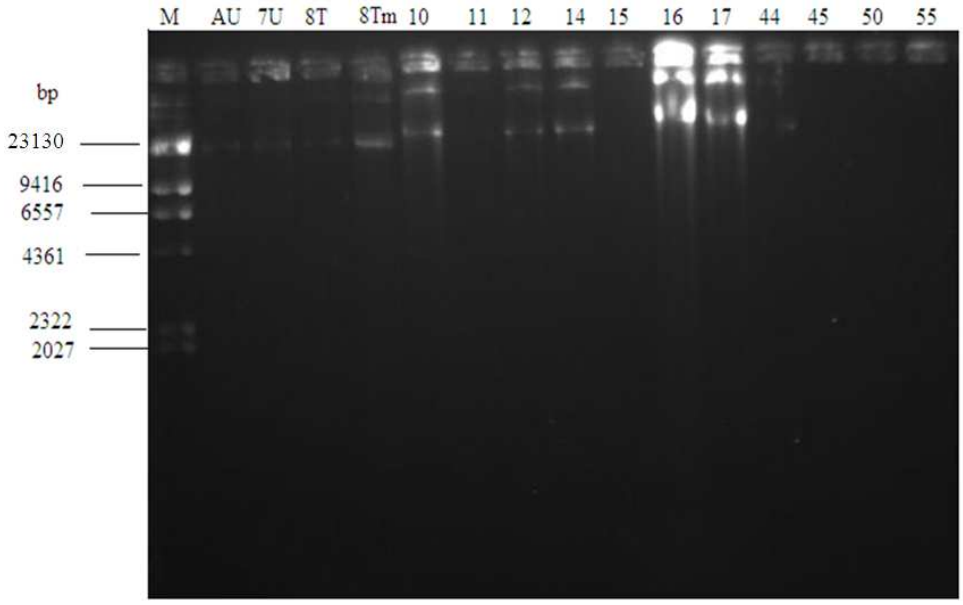

Fig. 2. Showing the plasmids of the *Acinetobacter* isolates M- Molecular ladder to serve as ruler for the procedure. The numbered and lettered wells represent different patient isolates. (AU, 7U, 8T, 10-55: Represent *Acinetobacter* isolates from different patients)

Prior to the introduction of current advanced resuscitatory devices, *Acinetobacter* spp were mainly recovered from the urinary tract however with the advent of mechanical ventilation most of the organisms are now being isolated from respiratory tract specimens. In this study, majority 12(86%) of the isolates were recovered from tracheal aspirate. This finding is similar to the 87% observed by Raka *et al*. (2004) but much higher than 46.5% reported by Popescu *et al*. (2011). The high rate of recovery from the respiratory tract may be due to the invasive procedures that are carried out in the respiratory tract in the process of maintaining the airway. The low recovery rate (1%) of *Acinetobacter* spp from the urinary tract is similar to the 1.6% rate reported in the NNIS study (Gaynes *et al*., 2005). This further confirms the popular report that *Acinetobacter* is no longer a common uropathogen.

Severe underlying diseases, invasive diagnostic and therapeutic procedures used in ICUs have been demonstrated to predispose patients to severe infections with *A. baumannii* (Jarousha *et al*., 2008). In recent years, *A. baumannii* has become an important pathogen especially in intensive care units. Persistence of endemic *A. baumannii* isolates in ICU seems to be related to their ability for long-term survival on inanimate surfaces in patient's immediate environment and their widespread resistance to the major antimicrobial agents (Oberoi *et al*., 2009). Our finding of *A.baumannii* as the major pathogen in this study is therefore not too surprising as this has been reported earlier by Joshi *et al*. (2006).

All the clinical isolates of *Acinetobacter* in the study harbor plasmids of different molecular sizes. Ten of the isolates were isolated at about the same period of time with 6(42.7%) having plasmid size in the 23.1kb band and exhibit similar patterns showing that they may have originated from the same clone and a signal to potential outbreak strain. Outbreaks of *Acinetobacter* infections are linked to contaminated respiratory equipment, intravascular access devices, bedding materials and transmission via hands of hospital personnel. *Acinetobacter* outbreaks have been traced to common-source contamination, particularly contaminated respiratory-therapy and ventilator equipment, cross-infection by the hands of health care workers who have cared for colonized or infected patients or touched contaminated formite and to the occasional health care worker who carries an epidemic strain (Raka *et al*., 2004).

5. CONCLUSION

In conclusion the ICU is responsible for providing life support services to patients from diverse specialties. The isolation of *Acinetobacter* among

critically ill patients in the ICU is a cause for concern. There is an urgent need for education of health care workers in the ICU on proper infection control practices. There is also a need for active surveillance for *Acinetobacter spp* in the ICU.

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