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Bacterial Air Microflora Isolates in Two Obstetrics and Gynaecology Units of A Hospital in Nigeria are Potential Threats of Nosocomial Infections.

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ABSTRACT

Ever since the history of infirmaries, nosocomial infections have been of grave threats to hospital set-ups, the deadliest being nosocomial respiratory tract infection (RTI). Nosocomial RTI was consequently investigated in two units of Obstetrics and Gynaecology department of a hospital in Nigeria using the "Settling Plate" technique and various culture media for bacteria isolation. Identification of the isolates was done on the basis of each isolate's cultural, morphological and biochemical characteristics. Six potentially pathogenic bacteria genera/species (*Staphylococcus aureus, Streptococcus* sp, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella* sp *and Bacillus* sp) were isolated in the two units. *Staphylococcus* was isolated in all (100%) of the exposed plates; *Streptococcus* sp *and Bacillus* sp. in 75% (6/8), *Pseudomonas aeruginosa* and *Escherichia coli* in 50% (4/8), and *Klebsiella* sp in only 25% (2/8). This work therefore indicated high degree of poor atmospheric sanitation in those units as well as strong indication of imminent threat of nosocomial respiratory tract infections. Routine sanitary surveillance and fumigation of the wards are advocated.

Keywords: Nosocomial Infection; Bacteria; Ward; Obstetrics and Gynaecology.

INTRODUCTION

Rajesh and Rattan (2008) defined nosocomial infection as those infections which developed during hospitalization and were not present at the time of admission into hospitals. Willey *et al.* (2009) referred to it as those infections resulting from pathogens acquired by patients while in hospital or other clinical care facilities. Besides affecting patients, recent advances showed that it may also affect nurses, physicians, aides, visitors, sales-persons, delivery personnel, custodian, as well as anyone else or passersby coming in contact with hospitals and its unhealthy environments. Meltzer (2003) indicated even possible spillover on nearby or whole community.

This type of infection has been an age-long headache of patients, to an extent, members of hospitals staffs too. Those caused by multi-drug-resistant microorganisms are presently the greatest growing problems in many health care institutions (Ginocchio, 2002). Most worrisome is the fact that in spite of advances in modern medicine, it still poses great risks of increased morbidity and mortality on a high percentage rate of up to 40% in hospitalized patients. Omololu-Aso (2011) put the estimate at about 10% in United Kingdom. Precise data on developing countries is limited (Rajesh and Rattan, 2008).

Those at greatest risks are medical personnel, which includes doctors, specialists, consultants, nurses, matrons, medical laboratory scientists, medical students, laboratory attendants, etc, because, in spite of their training, they live-in as well as work with the pathogens in the environment on daily basis. Those at gravest risk are the immunodeficient patients, as well as those at the extremes of life (the aged and neonates) and should be given the greatest

precautionary measures. High risk areas include: Intensive care units, Nursery units, Dialysis units, and Analysis unit (Castle, 1980). That was one of the reasons why Obstetrics and Gynaecology ward was chosen in this work because that was where neonates, one group of those at the extremity of life, could be found.

Bhattacharya (2005) indicated that the medium for contamination included the hands of these medical personnel, inanimate objects around the hospital, as well as some patients' and carriers' activities such as coughing, sneezing, loud talking, shouting, etc. Food, drinking water, and unhygienic drugs are also very efficient media. Recent works also implicated mobile phones of health care workers (Elkholy and Ewees, 2010; Amadi *et al.*, 2013).

Rajesh and Rattan (2008) indicated that the etiologic agents are grouped under various species and genera of bacteria, viruses, fungi and protozoa (They shouldn't have omitted flatworms and roundworms *in toto*). These etiologic agents vary from hospital to hospital, and in different geographical locations, depending on the type of infection as well as the environmental predisposing factors found in that particular area (Struelens *et al.*, 2004). Or, as should be more appropriately stated, I quote, "Each hospital has its own ecosystem and micro-society, where determinants of antibiotic resistance are quite specific and therefore effective solutions will need to be tailored to local epidemiological circumstances and resources," unquote. Notable too, is that for a nosocomial infection to occur, there must be a source of infection, transmission and transmitter of the etiologic agent, and a patient susceptible to that infection (Boyd, 1995).

Sources of this type of infection are grouped into exogenous and endogenous sources. The commonest exogenous sources are fomites like stethoscopes, bronchoscopes, pagers, pens, ball-point biros, even patients hospital charts and laboratory report forms, computer keyboards, ventilators, respiratory equipments, endoscopes, wash bowls, bed pans, patients' beds and clothing, catheters, etc (Wiley *et al.*, 2009; Brook *et al.*, 2007; Maley, 2000; Brandy, 2006; and Borer *et al.,* 2005). Other sources include air, food, water, dusts, antiseptic and ordinary lotions, as well as aerosols shedding via sneezing, coughing, talking loudly, laughing, etc from other patients or carriers as earlier indicated.

Infection control has many aspects which includes surveillance for disease among patients and healthcare personnel, and determination of the number and kind of viable microbes (or more precisely, pathogens) in the hospital environments. Indeed, successful nosocomial infection control requires the education and co-operation of all hospital's members of staff. To this end, there should be hospital-infection-control officers, infection-control committee, and laboratory members-of-staffs working together to solve cases of nosocomial infections, and preventing new cases. This should be imperative for all hospital set-ups. Most importantly, such routine surveillance method should be simple, cheap and fast, especially for underdeveloped countries and private hospitals with low economies.

We therefore carried out this research work aimed at using a simple, cheap and quick "Settling plate" technique to investigating the potential of' atmospheres of hospital wards as vehicles of air-borne pathogens known to cause nosocomial infections, and thereby create awareness on them as nosocomial infections medium. Thus, appropriate recommendations could as well be made.

MATERIALS AND METHODS

Sample Collection: Two units (Labour ward and Ante-natal clinic) of Obstetrics and Gynaecology (O and G) departments of the hospital were chosen for the sampling. The "Settling plate" technique was used in the isolation of the bacteria air microflora of medical importance. By this technique, duplicate sterile culture plates of blood,

chocolate and Cysteine-Lactose-Electrolyte-Deficient (CLED) agars were exposed for 15 minutes each at various locations in the two units of the ward and labeled appropriately. The various locations were under the bed, near the air conditioners and activated fans, and near the windows and doors. Duplicates of blood and CLED agar were exposed in Labour ward, while same of chocolate and CLED agars were in Ante-natal clinic, making a total of eight culture plate samples. Immediately after the required time, the plates were covered up and quickly transported to the laboratory for analysis.

Isolation: The exposed plates of chocolate agar (Oxoid), blood agar (Oxoid), Cysteine-Lactose-Electrolyte-Deficient [CLED] agar were thereafter incubated at 37^{o}C for 24 hours. Pure cultures of all the colonies of microbes that developed in the above media were obtained by sub-culturing the entire colonies singly in fresh chocolate, blood, and CLED agar and again incubated at 37^{o}C for 24 hours. At the end of various incubation periods, the plates were read, and the pure cultures obtained were transferred into agar slants, from where they were taken for biochemical tests.

Characterization and identification of isolates: The bacteria isolated were characterized based on microscopic appearances, colonial morphology, and biochemical reactions. The motility of the isolated bacteria was examined by the "hanging drop technique." Their Gram reactions and cell morphology were examined from heat-fixed smears. The microbes were identified by the methods after Cheesbrough (2003), Cowan and Steel (1974) and Bergey's Manual of Determinative Bacteriology (Buchanam and Gibbons, 1974) (Tables 1 to 8).

RESULTS

A total of six different genera (*Staphylococcus aureus*, *Streptococcus s*p, *Bacillus* sp, *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella* sp) were isolated in the two units of O & G wards of the hospital. The isolated microbes from Labour ward and Antenatal clinic were almost similar (Table 9). It was found that Labour ward grew five different types of genera while Antenatal units grew six. Except *Bacillus* sp, all of the bacterial isolates are known agents of nosocomial infections. *Staphylococcus aureus* was isolated in all eight (100%) of the

Table 1: Pooled morphological features of bacteria isolates in exposed blood agar plates in Labour ward

Isolates	Gran stain	Shape	Motility	Spore	Haemolysis	Probable genus
AB1	+	Cocci	-	-	.	*Staphylococcus*
AB2	+	Cocci	-	-	β	*Streptococcus*
AB3	+	Rod	+	+	-	*Bacillus*
AB4	-	Rod	+	-	α	*Pseudomonas*
Control	NA	NA	NA	NA	NA	No growth

Keys: + = Positive; - = Negative; NA = Not applicable

Table 2: Pooled biochemical reactions of bacteria isolates in exposed blood agar plates in Labour Ward

Genus	Catalase	Coagulase	Oxidase	H_2S	Optochin	V.P	Gelatin	O/F
Staphylococcus	+	+/-	-	+	-	+	-	F
Streptococcus	-	-	-	+	+	+	+	F
Bacillus	+	NA	-	-	-	-	-	F
Pseudomonas	+	NA	+	+	-	-	+	O
Control	NA	NA	NA	NA	NA	NA	NA	No growth

Keys: + = Positive; - = Negative; NA= Not applicable; O = Oxidative; F = fermentative VP = Voges Prokauer; α = Alpha haemolysis; β= Beta haemolysis

Table 3: Pooled morphological features of bacteria isolates in exposed CLED agar plates in Labour Wards

Isolates	Gram stain	Shape	Motility	Spore	Gelatin	Lactose	Probable genus
BC1	-	Short rod	+	-	-	+	*Escherichia*
BC2	+	Cocci	-	NA	-	NA	*Staphylococcus*
BC3	+	Cocci in chain	-	-	+	NA	*Streptococcus*
BC4	+	Rod	+	+	-	+	*Bacillus*
Control	NA	NA	NA	NA	NA	NA	No growth

Keys: - = Negative; += Positive; NA= Not applicable

Table 4: Pooled biochemical reactions of bacteria isolates in exposed CLED agar plates in Labour wards

Genera/species	Catalase	Coagulase	Oxidase	Citrate	VP	Optochin	Glucose	Indole
Escherichia coli	+	-	-	-	-	NA	Gas	+
Staphylococcus	+	+	-	NA	-	-	Acid/Gas	-
Streptococcus	-	-	-	NA	+	+	NA	NA
Bacillus	+	-	+	-	-	-	Acid/gas	-
Control	NA	NA	NA	NA	NA	NA	NA	NA

Keys: - = Negative; += Positive; VP = Voges Proskauer; NA= Not applicable

Table 5: Pooled morphological features of bacteria isolates in exposed chocolate agar plates in Ante-natal ward

Isolates	Gram	Shape	Motility	Gelatin	Lactose	Probable genus
CC1	+	Cocci	-	-	NA	*Staphylococcus*
CC2	+	Cocci in chain	-	+	NA	*Streptococcus*
CC3	-	Blue-green rod	+	+	-	*Pseudomonas*
CC4	-	Short rod	+	NA	+	*Escherichia*
Control	NA	NA	NA	NA	NA	No growth

Keys: - = Negative; += Positive; NA= Not applicable

Table 6: Pooled biochemical reaction of bacteria isolates in exposed chocolate agar plates in Ante - natal ward

Genera/species	Catalase	Coagulase	Oxidase	Indole	Citrate	VP	Optochin	Glucose
Staphylococcus aureus	+	+	-	-	NA	-	-	Acid/gas
Streptococcus	-	-	-	NA	NA	+	+	NA
Pseudomonas aeruginosa	+	NA	+	-	NA	-	NA	_
Escherichia	+	-	-	+	+	-	NA	Acid/gas
Control	NA	NA	NA	NA	NA	NA	NA	NA

Keys: - = Negative; += Positive; NA= Not applicable

Table7: Pooled morphological features of isolates in exposed CLED agar in Ante-natal ward

Isolates	Gram	Stain shape	Motility	Spore	Gelatin	Lactose	Probable genus
DC1	-	Short rods	+	-		+	*Escherichia*
DC2	+	Rods	+	+	-	+	*Bacillus*
DC3	-	Rods	-	-	-	+	*Klebsiella*
DC4	+	Cluster cocci	-	NA	-	NA	*Staphylococcus*
Control	NA	NA	NA	NA	NA	NA	No growth

Keys: - = Negative; += Positive; NA= Not applicable

Table 8: Pooled biochemical reactions of bacteria isolates in exposed CLED agar plates in Ante-natal ward

Genus	Citrase	Coagulase	Glucose	Citrate	indole	Urease	Oxidase	VP
Escherichia	+	-	Gas	-	+	-	-	-
Bacillus	+	-	Acid/gas	-	-		+	-
Klebsiella	+	-	Gas	+	-	+	-	+
Staphylococcus	+	+	Acid/ gas	NA	-	-	-	-
Control	NA	NA	NA	NA	NA	NA	NA	NA

Keys:+ = Positive; - = Negative; NA = Not applicable; VP = Voges Proskauer

Table 9: Microbial isolates in Labour and Ante -natal wards

Ward	Isolates
Labour	*Staphylococcus aureus* *Streptococcus* sp. *Bacillus* sp *Pseudomonas aeruginosa* *Escherichia coli*
Antenatal	*Staphylococcus aureus* *Streptococcus* sp *Pseudomonas aeruginosa* *Escherichia coli* *Bacillus* sp *Klebsiella* sp

Table 10: Percentage/ratio occurrence of isolates in the culture plates

Isolate	Ratio	Percentage (%)
Staphylococcus aureus	8/8	100
Streptococcus sp	6/8	75
Bacillus sp	6/8	75
Pseudomonas aeruginosa	4/8	50
Escherichia coli	4/8	50
Klebsiella sp	2/8	25

exposed plates,*Streptococcus* and*Bacillus* sp in 75% (6/8),*Pseudomonas aeruginosa* and *Escherichia* sp in 50% (4/8), while only *Klebsiella* sp was isolated in 25% (2/8) of the exposed plates (Table 10). Nothing grew in any of the control plates. The pooled morphological and biochemical features of the bacterial isolates in the units are as shown in Tables 1 to 8.

DISCUSSION

In this study, the isolation of six potentia pathogens in Labour ward and Ante-nata clinic of O and G department not only

indicated a high potential health hazard, as well as high degree of poor sanitation set up, but most importantly contamination with nosocomial pathogens and its serious implications. Except *Bacillus* sp, all of the bacterial isolates are known agents of nosocomial infections. Also, as an assessment of the level of hygiene in a hospital ward in conformity with the Law of the Ministry of Health (1995) in some countries, this work is in line with such regulations.

Most seriously stating this implication is the fact that among many risk factors (i.e. chemical, physical, biological and psycho-social) which can evoke many disorder and damage the health of patients and staff, Brook *et al.* (1995), Aghove *et al.* (1997) and Noskova (2004) indicated that the most risky is the microbiological factor in hospitals set-ups, especially for the immunocompromiseds. This is because it has the potential to start nosocomial infection. This literature is also aware that the so defined "micro-biological factor" is never given its pride of place in most hospitals, especially in third world countries; consequently this work was tailored along that highlight.

Further to that, Sobotova *et al.* (2006) indicated that respiratory tract infection (RTI) is the most prevalent among nosocomial infection (NI), with least in gastrointestinal NIs; so it was partly why this work focused this surveillance on RTI atmosphere, using "settling plate" approach. Reason for the stated highest incidence of RTI as nosocomial infection must be due to the fact that the air is the most contaminated factor in hospital set up as well as the most senselessly neglected, while food and drink (route of GIT infections) always have the highest hygiene.

The highest rate of contamination by *Staphylococcus aureus* (Table 10) was in conformity with the work of Omololu–Aso *et al.* (2011). However, result obtained in this work was a bit not in conformity with that of Sobotova *et al.* (2003) that varied from ward to ward, and that of Pittet *et al.* (2011) that varied with hospital locations. Further, in this work, except *Klebsiella* sp, microbes isolated were similar in both units of the O & G department.

Another fact to seriously consider is the high contamination in Labour Ward which should not have been the case because of the high vulnerability of newborns that could be found there. The higher number of isolates found in the Antenatal clinic must be due to the higher level of activities there which includes staffs and visitors' movements, unlike in Labour ward where there is always little or no tolerance of non-members of staff. In general, the high number of isolates found in both units (Labour ward and Ante-natal clinic) is not very surprising if judged by the high level of movements there by highly anxious relations, as well as the use of very poorly trained staffs as ward-maids, cleaners and orderlies in under-developed countries. Further, unrestricted and unwarranted tolerance of visitors and un-queried relations that even sleep-in with patients in the wards is another graver factor.

Indeed, with the increasing number of high-risk patients, particularly those with immune deficiencies or have complex medical and surgical problems, the susceptibility of those patients' class to infections, the trauma of acquiring new infections (strange or worst than the one that call for hospitalization), the tragedy of drug-resistant infections, the paucity of information and inadequate sanitation in third-world countries, etc, should be very worrying and even call for more emphatic highlights.

CONCLUSION AND RECOMMENDATION

This work indicated high degree of poor sanitation and strong index of potential or imminent nosocomial infections in the niches studied. Solution to the finding lies in developing active preventive policies and tactics like routine decontamination of hospital unit by fumigation. In addition, development of antimicrobial aerosols such that should be effective against spores, to sanitized airborne microbes, would also

greatly help. Another approach is strict guideline as in the new Infection Control Nursing Association [ICNA] (2002) that must not only be issued or decreed but must also be seen strictly complied to. Lastly, with a simple, cheap and fast routine surveillance technique, such as this work, hospital set-ups should have no further excuse, of even low economy, for senseless negligence of nosocomial infection control.

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