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Clinical bacterial isolates from hospital environment as agents of surgical wound nosocomial infections

Razaq F. Atata[1*], Yakubu K. E. Ibrahim[2], Philip F. Olurinola[2], Abdulganiyu Giwa[3], Aliu A. Akanbi II[4] and Ali A. Sani[5]

[1]Department of Microbiology, Faculty of Science, University of Ilorin, PMB 1515, Ilorin. Nigeria. [2]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Nigeria [3]Department of Clinical Pharmacy and Pharmacy Administration, Faculty of Pharmacy, University of Maiduguri. Nigeria. [4]Department of Medical Microbiology and Parasitology, College of Health Sciences, University of Ilorin, PMB 1515, Ilorin. Nigeria. [5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri. Nigeria

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Abstract
The relationship between bacteria isolated from the hospital environment and those from wounds of operated patients was investigated to determine the causal agents of surgical site nosocomial infections. The study was carried out on bacterial species isolated from the theatre, surgical ward and patients' surgical wounds in a tertiary health institution in Nigeria. Bacteria were isolated from the air, floor and patients' surgical wounds in the theatre and surgical ward by using MacConkey agar, Chocolate agar Nutrient agar and Peptone water broth as isolating media. Plasmid sizes and bands of selected twenty (20) of the isolates were determined by electrophoresis analysis to determine their relatedness. The bacterial species isolated were: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Proteus mirabilis, Proteus vulgaris, Citrobacter freundii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Serratia marscenses*. The result of electrophoresis showed that some of the isolates from the hospital environment and surgical sites have the same number of bands and molecular weight. It was concluded that isolates from the hospital environment with the same numbers of bands and molecular sizes with those isolated from patients wounds in the same hospital environment are of the same strain, and must have come from the same source, and therefore are likely to be responsible for the surgical wound infections observed in the patients studied.

Keywords: Surgical wound infections, Environmental pathogens and nosocomial infections, Hospital pathogens.

Introduction

Environmental factor may either assist or impede the transmission of microorganisms. The environment may be conducive to growth and survival of the organism. Health care settings are an environment where both infected persons and persons at increased risk of infection congregate (Mayon-White, 1988). Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for patients and staff. Patients who get infected in the hospital are a further source of infection (Haley *et al*., 1985).

Crowded conditions within the hospital, sharing of equipments or frequent

* Corresponding author. *E-mail address*: rfatata@yahoo.com or rfatata@unilorin.edu.ng Tel: +234 (0)8035036850
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transfers of patients from one unit to another, and concentration of patients highly susceptible to infections in one area (e.g. newborn infants, burn patients, intensive care) may increase the chances of exposure to the microbial flora of other patients there by contribute to the development of nosocomial infections (Kirkland, 1999). Microbial flora may contaminate objects, devices, and materials which subsequently contact susceptible body sites of patients.

Respiratory viruses and rotaviruses persist for prolonged periods on inanimate objects, and transmission of RSV from inanimate environment has been documented (Bean *et al.,* 1982; Brady *et al.,* 1990; Hall *et al*., 1980; Hendley *et a*l., 1973; Ward *et al*., 1991; Keswick *et al*., 1983; Hall. 1981). Bedside tables and bedrails may be contaminated by Vancomycin resistance enterococci –VRE (Boyce *et al*., 1995; Lior *et al.,* 1996) and Methicillin Resistant *Staphylococcus aureus*-MRSA (Boyce *et al.,* 1997). Call bell may become contaminated by VRE (lLior *et al.,* 1996).

Environmental contamination may be important in transmission of relatively hardy bacteria such as enterococci (Boyce *et al*., 1994) and spore-forming bacteria such as *Clostridium difficile* (Gerding *et al.,* 1995; Johnson and Gerding, 1998). The mobil equipment that is shared among patients may be source of transmission. For instance, transmissions of VRE and C. difficile have been linked to contaminated thermometers (Livornese *et al*., 1992; Porwancher *et al*., 1997; Brooks *et al*., 1998; Jernigan *et al.,* 1998). Contaminated blood pressure cuffs have been implicated in transmission of *C. difficile* (Mania *et al*., 1996) and Klebsiella (Myers, 1978). Recent studies have shown that stethoscope diaphragms and otoscope specula become contaminated with microorganisms including MRSA (Smith *et al*., 1996; Cohen *et al*. 1997; Dias *et al*., 1997; Wright *et al*. 1995; Jones *et al.,* 1995).

Many patients care items including bedside table and bed rails, may not be cleaned routinely. Although there have been much concern about the most appropriate cleaning agents, the actual method of cleaning may be more important than the agent used (Bonilla *et al*., 1997; Jackson and Lynch, 1996; Rutalla *et al.,* 1997). The environment may play a larger role in transmission of certain pathogens than previously appreciated, reinforcing the importance of minimizing environmental contamination by patient excretions and secretions, avoiding unnecessary hand contact with environmental surfaces, ensuring adequate resources for procurement of necessary items (2006).

This work therefore aimed at verifying the relationship between bacterial isolates from the hospital environment and those isolated from the patients' surgical wounds.

Experimental

Bacteriological study of theatre air. The bacterial load of the air in the operation theatre of a University Teaching Hospital in Nigeria was studied using settling plate method. Plates containing Nutrient agar, MacConkey agar, and Chocolate agar were exposed and placed in different areas of the theatre at the start of each surgical operation and left exposed until the operation procedure was completed. Thereafter, they were covered and taken to the laboratory for incubation. The duration for each operation was recorded. The Nutrient and MacConkey agar plates were incubated at 37^{o}C in an ambient incubator while plates containing Chocolate agar were incubated in a carbon dioxide incubator containing 10% CO_2 for 24 hrs. Colonies that developed after 24hr of incubation in the plates were enumerated and recorded. Based on the number of colonies per plate, the number of exposed plates and the duration of the surgical operation, the number of colonies in the air per hour was determined and recorded number of colonies

forming unit per cubic meter of air (cfu/m^3). Colonies showing different morphological characteristics were isolated, purified into pure cultures, coded, sub-cultured onto nutrient agar slant and kept in refrigerator for subsequent characterization.

Bacteriological study of theatre floor. While the operation was in progress, the bacteria load of the theatre floor was studied using swabbing method. The floor of theatre which has already been demarcated (one meter square) was sub divided into 16 sub-squares, and one of these squares was randomly chosen and swabbed using sterile cotton swab moistened with sterile normal saline. The swab was then put into test tube containing 9ml sterile normal saline and mixed properly as to discharge it contents. One milliliter (1ml) each of this mixture was then used to flood plates of Nutrient agar, MacConkey agar and Chocolate agar. Plates of nutrient agar and MacConkey agar were incubated at 37^{o}C for 24 hrs while the plates of Chocolate agar was incubated under 10% CO_2 for 24 hrs (Bond and Schulster, 2004 and Favero *et al*., 1984). The colonies that developed after 24 hrs of incubation, were counted, streaked onto nutrient agar slants and stored at 7^{o}C in refrigerator for further processing.

Bacteriological study of operated surgical sites. Surgical operations in the theatre were followed and the time taken to complete each surgical operation cycle (i.e. time between incision of the operation site and closure of sutured sites) was recorded. Immediately after completion of an operation and closure of the operated site, it was swabbed using sterile swab stick. The swab was then inoculated into 9ml sterile peptone water from which 1ml each was inoculated on Nutrient agar, MacConkey agar and Chocolate agar plates. The plates were taken to the Microbiology Laboratory of the Hospital, incubated aerobically (for Nutrient and MacConkey agar plates) or under 10 % CO_2 (in case of Chocolate agar plates) at 37^{o}C for 24 hours. The colonies that that developed after incubation were sub-cultured onto nutrient agar slant and stored in refrigerator for further characterization.

Bacteriological study of patient's environment in the wards. The microbial loads of the air environment in the Wards to which the patients were transferred to after the surgical operations were also evaluated using the plate settling method as described above. The microbial levels of the floor of the Wards where the operated patients were kept were also determined using cotton swabs as discussed above. Resulting colonies were computed and sub-cultured onto nutrient agar slants, kept in refrigerator at 7^{o}C for further characterization.

Detection of plasmids and determination of their sizes in the resistant isolates. This test essentially involves application of biotechnology tools. It is in several stages. It was carried out in the laboratory of the Nigeria Medical Research Institute (NIMR), Oshodi, Lagos.

Culture purification: Purity of bacterial cultures to be used for the test was ascertained by sub-culturing the isolates on Nutrient and MacConkey agars.

Plasmids separation: A twenty four hours (24 h) old culture of isolates was inoculated in 5ml of lauryl tryptose broth (Oxoid) and incubated at 37^{o}C for 18 hrs. The culture was spun at 4000 rpm for 10 minutes using laboratory centrifuge (model SM800B). The supernatant was discarded leaving the pellet. The pellet was further vortexed with little quantity of supernatant in a Stuart vortex mixer at 2,500 rpm for few second.

The mixture was emptied in Eppendorf tubes which have already been labeled with the name of each isolates. The Eppendorf tubes with its contents were further centrifuged at 11,000 rpm for 5 minutes and the supernatant discarded. With a little quantity of the supernatant, the suspended pellet was again vortexed. Using automatic

pipette, excess supernatant was removed and the pellet immersed in pack of ice block. Thereafter, 150µl of 3M sodium acetate of pH 5.2 was added, and allowed to stand in ice pack for 5 minutes. The mixture was vortexed and further spun for 2 minutes in a micro-centrifuge to pellet out cell debris and chromosomal DNA.

The supernatant was removed to fresh Eppendorf tubes, and then 0.9ml of 100% ethanol which has been pre cooled to -20^{o}C, was added and vortexed for 2-5 minutes, spun again at 11,000rfm for 2 minutes to pellet out plasmid DNA and RNA. The supernatant was discarded and the pellet was rinsed twice with 1.0 ml of 70% ethanol. The pellet was dried under vacuum for 3 minutes, re-suspended in 20µl of (TE) buffer for the electrophoresis stage. When not immediately used, the buffered pellet was kept in Freezer at -7^{o}C.

Electrophoresis: A 50ml of 0.8% w/v of agarose gel was prepared and 2 drops (7µl) of ethidium bromide was added. The mixture was poured in an electrophoresis chamber with comb in place and allowed to set. After the gel had set, the comb was removed thereby creating wells on the starting point of the gel. Twenty microliter (20µl) of the sample was mixed with 2µl of bromophenol blue as the tracking buffer. The mixture was loaded into each well for each sample. A 20µl of HIND II DNA molecular weight marker was also mixed with 2µl of bromophenol blue, and loaded in first well to serve as standard for calculating the molecular weight of isolated plasmids. After loading the electrophoresis chamber, it was connected to a transformer and plugged to source of electricity. The electrophoresis gel was run at a voltage of 65-70 Volts for 1½ hr using (TBE) as running buffer. After 1½hr of running the electrophoresis, the chamber was disconnected and taken to dark room for photographing and measurement of distance of migration of the DNA plasmids extracted from the isolates and the standard marker. The gel was emptied on the stage glass and with illumination by ultraviolet light (Plate 1.0); different migrations levels of each plasmid were shown. These were photographed using a Polaroid camera attached to the photographing machine (plate 1). After photographing, distance of migration of each isolate plasmid and that of the marker was measured using metric ruler and recorded.

Determination of Molecular Weight of Plasmids of the Isolates: Using the distance of migration of the bands (plasmid unit) in each isolate and matching the value of the marker in the standard with it, the plasmid sizes of the isolates were determined.

Determination of Sources of the Isolated Nosocomial Pathogens: Based on the morphological and biochemical characteristics of the isolate, the isolates were identified to species level. With this identification to species level, antibiotic resistance patterns and plasmid profiles, of isolates from the floors and air of theatre and wards were matched with those isolated from the surgical sites. Similarities in at least two of the three characteristics were taken as being indicative of the organism coming from the same source.

Results

Table 1 shows monthly bacterial levels of environmental air and floors of the theatre and surgical ward of a University Teaching Hospital in Nigeria 2007. Bacterial counts from the air of the theatre ranged from 3.0cfu/m^3 (April) to 11.0 cfu/m^3 in August. Similarly, bacterial counts from the floors of the ward ranged from 2.1 x 10^2 cfu/m^2 to 4.61x10^3 cfu/m^2. Bacterial count from the wards ranged from 5 cfu/m^3 to 29cfu/m^3 (Table 1).

Table 2 shows the distribution of bacterial species during operations in the theatre and monitoring of patients in the surgical wards (2007). *S. epidermidis,*

Klebsiella spp, and *S. aureus* were isolated in the air, floor and wound of patients in both the theatre and surgical ward. In surgical ward *Ps. aeruginosa* was isolated in the air, floor and the patients' wounds. Streptococcus was also isolated from the wound, though it was not isolated from the air and floor of the ward (Table 2).

Table 3.0 shows comparisons of number and sizes of isolates' plasmids from air, floor and wounds of patients in theatre and wards of the teaching hospital. Out of 18 multiple antibiotic resistant isolates subjected to agar gel electrophoresis analysis, 17 had plasmids, with number of band ranging from 1 to 3. Some of the isolates have the same number of plasmid bands and sizes (Table 3). All isolates except two had one plasmid common to them in size.

Plate 1, shows different plasmid bands exhibited by some of the antibiotic-resistant isolates. In plate 1.0, isolates on lane 2, 5 and 6 have the same plasmid sizes and number. Isolates on lane 3 and lane 8 also have the same number of plasmids and size.

Plate 2 shows different plasmid bands exhibited by some of the antibiotic-resistant isolates. In plate 2 isolates on lane 11, 13 and 14 have the same number of plasmids and the same plasmid sizes. Similar observation was made on isolate on lane 17 and 19.

Discussion

There have been doubts about the role of the hospital environment in development of nosocomial infections in patients; and therefore underplay the importance of chemical disinfection of the hospital surfaces. The general agreement is that environment did not contribute any significant effect in causation of nosocomial infections. The results of this work however, showed that indeed, endemic hospital bacterial isolates are among organisms responsible for surgical site nosocomial infections in our hospitals. The result of this work, though showed that bacterial load in the floor and air of the theatre and surgical ward were below infectious does of 10^6 cfu/m2, but pathogenicity of organisms depends not only on number of organisms but also on virulence and invasiveness of the pathogens, and resistance of the patients. Patients receiving treatment in hospital are likely to have low immunity and as such susceptible to microbial infections.

Table 1 Monthly Bacterial Levels of Environmental Air and Floors of the Theatre and Surgical Ward of a University Teaching Hospital in Nigeria 2007

Month	Microbial Count			
	Theatre		Ward	
	Floor (cfu/m^2)	Air ($cfu/m^{3)}$)	Floor (cfu/m^2)	Air (cfu/m^3)
January	$3.30 \times 1\ 0^2$	8	2.18×10^2	7
February	2.40×10^2	5	2.90×10^3	9
March	3.70×10^2	6	4.16×10^3	12
April	4.17×10^3	3	2.99×10^3	5
May	3.99×10^4	9	4.10×10^2	14
June	2.71×10^3	6	2.82×10^3	21
July	3.34×10^4	5	8.15×10^2	16
August	4.55×10^3	11	7.30×10^3	10
September	5.23×10^3	7	3.20×10^3	29
October	3.13×10^2	10	5.21×10^2	15
November	2.45×10^3	8	4.61×10^3	18
December	1.30×10^3	6	4.16×10^3	9

Table 2 Distribution of bacterial species during operations in the theatre and monitoring of patients in the surgical wards (2007).

Isolate	Theatre			Ward		
	Air	Floor	Wound	Air	Floor	Wound
S. epidermidis	+	+	+	+	+	+
Klebsiella sp.	+	+	+	+	+	+
Bacillus subtilis	-	+	-	-	+	-
Enterobacter sp.	-	+	+	-	-	-
Serratia marcescens	-	+	-	-	+	-
Pseudomonas sp.	+	-	-	-	-	-
E. Coli	-	+	-	+	-	-
Ps. aeruginosa	+	+	-	+	+	+
Serratia sp.	+	-	-	-	-	-
Bacillus cereus	+	-	-	+	+	-
Bacillus megaterium	+	-	-	+	-	-
Citrobacter freundii	-	-	-	-	+	-
Proteus mirabilis	+	+	+	+	+	-
Bacillus sp.	-	+	+	-	+	-
Streptococcus spp.	+	-	-	-	-	+
Staph. Aureus	+	+	+	+	+	+

Table 3 Comparisons of number and sizes of isolates' plasmids from air, floor and wounds of Patients in Theatre and Wards of the teaching hospital.

Isolates	Sites sampled	No. of Plasmids	Plasmids sizes (Kbp)
S. epidermidis	Theatre Air	2	23.130, 27.306
S. epidermidis	Theatre Wound	3	16.962, 84.744, 20.462
S. epidermidis	Theatre air	2	23.130, 54.9267
S. epidermidis	Theatre wound	1	23.130
S. epidermidis	Ward floor	1	23.130
S. epidermidis	Ward floor	2	23.130, 54.9267
S. epidermidis	Ward wound	3	23.130, 39.2333, 42.785
S. epidermidis	Ward wound	2	23.130, 54.9267
S. aureus	Ward air	2	23.130, 59.47714
S. aureus	Theatre air	1	23.130
S. aureus	Theatre floor	1	23.130.
S. aureus	Ward wound	2	23.130, 59.47714
Strept. pyogen	Theatre air	3	27.756.
Pr. mirabilis	Theatre floor	2	23.130, 25.894
Pr. mirabilis	Theatre wound	2	23.130, 25.894
Pr. mirabilis	Theatre air	1	23.130
Pr. mirabilis	Ward air	1	23.130
Ps. aeruginosa	Ward wound	2	15.420, 22.5984
Kl. pneumonia	Theatre air	1	23.130
Bacillus cereus	Ward floor	nil	Nil

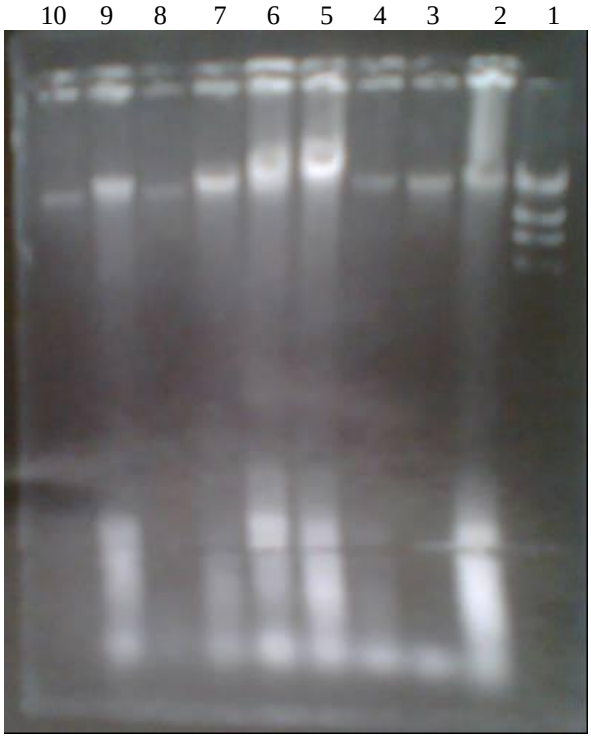

Plate 1. Different Plsmid bands exhibited by some of the antibiotics resistant isolates.
1. Marker standard 2. *Staphylococcus epidermidis* 3. *Proteus mirabilis* 4. *Proteus mirabilis*
5. *Staphylococcus epidermidis* 6. *Staphylococcus epidermidis* 7. *Pseudomonas aeruginosa*
8. Proteus mirabilis 9. *Staphylococcus epidermidis* 10. *Klebsiella pneumoniae*

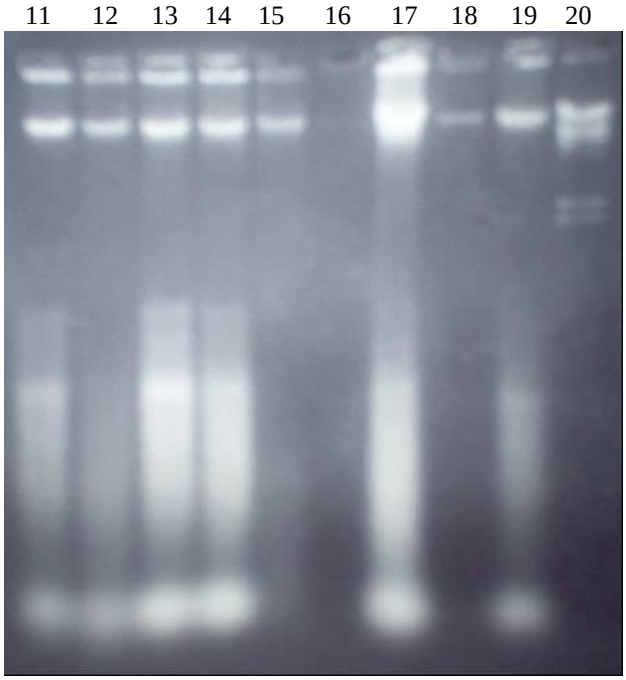

Plate 2. Different plasmid bands exhibited by some of the antibiotic resistant isolates.
11. *Staphylococcus aureus* 12. *Streptococcus pyrogenes* 13. *Staphylococcus aureus*
14. *Staphylococcus aureus* 15. *Staphylococcus aureus* 16. *Bacillus cereus*
17. *Proteus mirabilis* 18. *Staphylococcus epidermidis* 19. *Proteus mirabilis* 20. Marker standard.

Whyte *et al*., (1991) reported that wound contamination, as shown by intra-operative culture, is associated with later wound infection and that during cholecystectomy, the number and species of bacteria cultured from bile are predictive of wound contamination and later wound infection. Garibaldi *et al*., (1991) found that 30 or more colony-forming units (CFU) of bacteria cultured from a wound are predictive of wound infection, regardless of wound class.

The isolated bacterial species include; *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyrogenes, Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Proteus mirabilis, Proteus vulgaris, Citrobacter freundii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Serratia marscenses*. All of these isolates except *Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Citrobacter freundii and Serratia marscenses* have been documented as causative agents of surgical site nosocomial infection (Onche and Adedeji 2004 and Bowler *et al*., 2001).

Comparison of plasmid sizes and numbers of some selected isolated bacteria from surgical sites and floor and air of the Hospital Theatre and surgical ward showed that some of the isolates have plasmid band of the same number and sizes which indicated that they are of same origin, most probably the hospital environment. The plasmid profiling also showed that two isolates of *S. aureus* (one isolated from surgical ward and the other from a patients wound site in the ward) had identical plasmid band and sizes. This therefore established a linkage between environmental contamination and nosocomial infection particularly of surgical sites.

These findings were supported by the report of Cozad, et al., (2003). It is however in contrast with submission made by Eickhoff (1970); American Hospital Association Committee on infection within the Hospital, (1974); Rafferty and Pancoast, (1984) who concluded that there is no association between health care associated infection rate and microbial contamination of air or environmental surfaces.

Based on the above reported findings, we conclude that organisms from the hospital environment are important factor to be considered in control of nosocomial infections in hospitals as the isolated bacterial species from the surgical wound have been established to be of the same origin with those isolated from the air and floor of the theatre and surgical wards. The implication of these findings is that contamination of surgical wound and hence the infections of the wound arise from organisms from the hospital environment. Therefore chemical disinfection of hospital environment is among the necessary measures that must be strictly observed.

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