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Original Article

Screening for some virulent factors among bacterial isolates from surfaces of hospital fomites and hands of healthcare workers

Musa Yakubu Tula[1,*], Joel Filgona[2], Serah Erold Kyauta[3], Richard Elisha[1]

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ABSTRACT

The ability of the bacterial isolate to cause debilitating effects on the host is intricate and is a function of many factors, particularly that of the host and the bacteria. Among the bacterial factors are the virulence mechanisms. As such this research was a cross-sectional study conducted between October–December 2021 to establish the existence of virulent determinants on bacterial isolates from hospital fomites and the hands of healthcare workers. To achieve this, 100 samples (including sink, beddings, door handles, benches, and hands of healthcare workers) from children, female and male wards of Mubi General Hospital were analyzed for bacterial growth and were identified by standard procedure. Isolates were subsequently screened for virulent determinants (hemolysis, hemagglutination, biofilm production, and heteroresistance) phenotypically by standard methods. From the 72 bacterial isolates recovered, 23(31.9%) were biofilm-producing organisms. Of these, 15(20.8%) and 8(11.1%) were moderate and high biofilm-producing organisms respectively with no statistical difference (P=0.665). *Pseudomonas aeruginosa* (13.9%) was the most predominant biofilm-producing organism. Furthermore, hemolysin production was predominant in *Staphylococcus aureus* (71.4%), while positive hemagglutination reaction was predominant in *P. aeruginosa* (38.5%). Sixteen (16) bacterial isolates showed heteroresistance (HR) to various antibiotics; of these, *Escherichia coli* (43.8%) constitute the majority of the isolates. The expression of such virulence determinants by bacterial isolates in the study area may constitute a health risk to patients and hamper the quality of health care delivery.

1. Introduction

In developing countries like Nigeria, bedridden patients in hospitals are more likely to acquire hospital-associated infections (HAIs), particularly the ones caused by bacteria. This could be attributed to inadequate decontamination procedures as a result of many factors notably limited resources. As such, the dissemination of HAIs may occur easily and sometimes facilitated by the hands of healthcare workers and surfaces of fomites in the hospital wards [1].

Bacteria being ubiquitous are unique microorganisms that can be found on or in the human body imparting valuable functions. Others, however, can inhabit, attack, and initiate injury to the host and are cataloged as

[1]*Department of Biological Science Technology, Federal Polytechnic Mubi, PMB 035 Mubi, Adamawa State, Nigeria*
[2]*Department of Microbiology, Adamawa State University, Mubi, Adamawa State, Nigeria*
[3]*Department of Biomedical and Pharmaceutical Technology, Federal Polytechnic Mubi, Adamawa State, Nigeria*
***Corresponding Author:** Musa Yakubu Tula (birtytv@gmail.com)*

disease-causing organisms. Species of bacteria that are pathogenic acquire an assortment of factors that enable them to inflict injury to their host and can also modulate these pathogenic determinants to withstand the host defence mechanisms [2]. However, the ability of the bacterial isolate to cause a debilitating effect on the host is intricate and is a function of many factors, particularly that of the host and the bacteria. The bacterial factors are the virulent determinants that help the bacterium inhabit the host. These factors may function as either secretory, membrane-associated, or cytosolic, which are either chromosomally encoded or carried on transferrable hereditary factors like plasmid [3, 4].

The understanding of the nature of bacterial pathogens, including their virulent determinants, is fundamental in the advancement of innovative therapeutic molecules and strategies to combat microbial infections, especially among the incapacitated in hospitals. This is of meticulous significance considering the widespread occurrence of novel organisms that are resistant to readily available antimicrobials [5]. For this reason, this research was conducted to establish the existence of virulence determinants on bacterial isolates from hospital fomites and the hands of healthcare workers in Mubi general hospital.

2. Materials and methods

2.1. Study Area

The study area was Mubi general hospital. Mubi General Hospital is located in Mubi-South LGA of Adamawa State within the coordinates 10°15'54.9"N 13°16'10.0" E

2.2. Sampling technique/collection

Hundred (100) non-clinical samples from the sink, beddings, tables, door handles, and hands of healthcare workers were collected randomly from the Mubi General Hospital environment. The samples were collected by means of sterile swab sticks and each was immediately introduced into Salmonella-Shigella agar, and repeated in MacConkey agar, Eosin Methylene Blue agar, Cetrimide agar, and Mannitol salt agar. After the incubation for 24 hrs, the pure isolates were transferred aseptically into nutrient agar slants and kept at refrigeration temperature for further use.

2.3. Bacteria Identification

After Gram-staining, each Gram-negative bacterial isolate was identified phenotypically on the Microgen A kit as shown in figure 1a [6]. However, *Pseudomonas aeruginosa* was identified based on its reaction to the cetrimide agar plate (Figure 1b). *Staphylococcus aureus* was identified by its Golden yellow colony on Mannitol salt agar, in addition to catalase and coagulase tests.

Fig. 1. a)Microgen GN A kit for identification of Gram-negative bacteria, b)*P. aeruginosa* coloration on cetrimide agar

2.4. Biofilm Formation

Biofilm formation among the bacterial isolates was determined by the tube method. In this method, each 24 hrs culture of bacteria in a tube of trypticase soy broth was emptied and washed with phosphate buffer saline. The tubes were air-dried and blotted with crystal violet for 20 min, after which the tubes were rinsed of excess stain with distilled water. Each tube was air-dried in an inverted manner. Positive biofilm development was documented when a visible purple layer covered the bottom of the tube. The isolates were categorized into non-biofilm, moderate, and high biofilm-producing organisms respectively when there is no visible film lining, medium intense film lining and intense film lining [7].

2.5. Preliminary detection of heteroresistance

The preliminary detection of heteroresistance to all the bacterial isolates was evaluated using agar disk diffusion

method [8] for the following antibiotic disks; septrin (30µg), imipenem (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), erythromycin (30 µg), ciprofloxacin (10 µg), amoxicillin (30 µg), amoxicillin-clavulanic acid (30 µg), gentamycin (10 µg), pefloxacin (30 µg), cefoxitin (30 µg), and streptomycin (30 µg). For each isolate, each disk was carefully laid on the surface of already inoculated Mueller-Hinton agar (MHA) plates. Following growth for 18-24hrs at 37°C in an incubator, the presence of discrete varied colonies within the clear inhibition zone was documented as heteroresistance to the tested antibiotic [9].

2.6. Hemolysin production

Hemolysin production was detected using blood agar (Nutrient agar augmented with sheep red blood cells). All bacterial isolates were grown on blood agar and incubated at 37°C for 24 hours. The organisms were classified as either α, β or γ-hemolytic. Complete lysis of RBC shown by clear zone around the colonies was taken as β-hemolysis. The presence of a halo (greenish colouration) around the bacterial growth was taken as α-hemolysis (partial hemolysis), while γ-haemolysis was documented when there was normal growth without changes in the culture medium (no hemolysis)[10].

2.7. Haemagglutination Test

The test was carried out based on the principles of RBC clumping in the presence of D-mannose as a result of bacterial fimbriae. The test was achieved by the direct bacterial haemagglutination test-slide method. Each isolate was introduced into nutrient broth in a test tube and incubated aerobically for 48 hrs at 37°C. Afterwards, 5 ml of RBC from human blood group O was dispensed into a test tube, washed thrice with normal saline, and reconstituted to a 3% suspension in the saline. The RBC suspension was either use immediately or within a week when kept at refrigerated temperature. One drop each of the broth culture of the test isolate and the reconstituted RBC suspension were positioned and mixed on a glass slide. After whirling the mixture gently for about 5 minutes, it was monitored for the presence of clumbing, which indicates a positive haemagglutination test. [11, 12].

2.8. Statistical analysis

Non-parametric Mann-Whitney statistics was used to evaluate the significant difference between biofilm and non-biofilm producing organisms, between moderate and high-biofilm producing organisms, and between hemagglutination and hemolysin producing organisms. The significant level was recorded at 95% confidence interval. All statistical analysis was carried out using SPSS version 17.0.

3. Result

The results in Table 1 showed the prevalence of bacterial isolates from hands of healthcare workers and inanimate surfaces in the hospital environment. From the 72 bacterial isolates recovered, 49(68.1%) and 23(31.9%) were non-biofilm and biofilm-producing organisms respectively. Of these, 15(20.8%) and 8(11.1%) were moderate and high biofilm-producing organisms respectively. The result further showed that biofilm was produced by *Escherichia coli, Pseudomonas aeruginosa, Providencia rettgeri,* and *Staphylococcus aureus* in different proportions. *Pseudomonas aeruginosa* was the most predominant biofilm-producing organism, closely followed by *Escherichia coli* with a prevalence of 13.9% and 8.3% respectively. However, none of the isolates of *K. pneumoniae, Citrobacter diversus, Shigella* spp., and *S. marcescens* produced biofilm.

Table 1. Biofilm-producing bacterial isolates from hand of healthcare workers and hospital fomites

Isolate	No (%)	Non-biofilm producing	Moderate biofilm producing [a]	High biofilm producing [a]
Escherichia coli	15(20.8)	9(18.4)	4(26.7)	2(25.0)
Klebsiella pneumoniae	3(4.2)	2(4.1)	1(6.7)	0
Pseudomonas aeruginosa	16(22.2)	6(12.2)	6(40.0)	4(50.0)
Citrobacter diversus	4(5.6)	2(4.1)	2(13.3)	0
***Shigella* spp**	2(2.8)	2(4.1)	0	0
Providencia rettgeri	4(5.6)	2(4.1)	1(6.7)	1(12.5)
Serratia marcescens	2(2.8)	2(4.1)	0	0
Staphylococcus aureus	26(36.1)	24(49.0)	1(6.7)	1(12.5)
Total	72	49(68.1)	15(20.8)	8(11.1)

Legend: parameters with same superscript typified lack of significant difference (P=0.665)

Non-biofilm-producing organisms were arithmetically higher than biofilm-producing

organisms but with no statistical difference (P=0.748). In the same vein, the number of moderate and high-biofilm-producing organisms showed no statistical difference (P=0.665)

The distribution of bacteria isolates for hemolysis and hemagglutination tests was documented in Table 2. The result showed that 14 (19.4%) of the bacterial isolates produced hemolysis on erythrocytes. From these, *Staphylococcus aureus* (71.4%) was the predominant strain with hemolysis production, followed by *Pseudomonas aeruginosa* (14.3%), and the least were *Escherichia coli* and *Serratia marcescens* with 1.7% prevalence rate each.

Table 2. Frequency of Bacterial isolates that are positive haemolysin and hemagglutination

Isolate	No (%)	No (%) + Haemolysis[a]	No (%) + Hemagglutination[a]
Escherichia coli	15(20.8)	1(7.1)	2(15.4)
Klebsiella pneumoniae	3(4.2)	0	3(23.1)
Pseudomonas aeruginosa	16(22.2)	2(14.3)	5(38.5)
Citrobacter diversus	4(5.6)	0	1(7.7)
***Shigella* spp**	2(2.8)	0	2(15.4)
Providencia rettgeri	4(5.6)	0	0
Serratia marcescens	2(2.8)	1(7.1)	0
Staphylococcus aureus	26(36.1)	10(71.4)	NT
Total	72	14(19.4)	13(18.1)

Legend: parameters with the same superscript typified lack of significant difference (P=0.508)

For hemagglutination (HA), only 13 bacterial isolates were HA positive representing a prevalence of 18.1%. from these, the highest was from *P. aeruginosa* (38.5%), while the least was from *Citrobacter diversus* (7.7%). However, all isolates of *Providencia rettgeri* and *S. marcescens* were HA-negative.

The result from Table 3 showed the heteroresistance profile of bacterial isolates and sources from where they were isolated. The majority of the bacteria that exhibit heteroresistance were isolated from tables (43.8%), sinks (25.0%), beddings (18.8%), and the least from door handles (6.3%) and hands of healthcare workers (6.3%). *Escherichia coli* was heteroresistant to 1-2 antibiotics, mostly amoxicillin-clavulanic acid (amc) and ceftazidime (caz). *Pseudomonas aeruginosa* was heteroresistant to 1-7 antibiotics, mostly, imipenem (imp) and ceftazidime (caz). *Shigella* spp., showed heteroresistance to 7 antibiotics, while *Serratia marcescens* showed heteroresistance to 8 antibiotics.

The result in Table 4 showed the frequency of bacterial isolates that were heteroresistant. The result showed that 16 bacterial isolates portrayed heteroresistance to one or more antibiotics which constitutes a prevalence of 22.2%. Of these, *Escherichia coli* constitute the majority of the isolates with a prevalence of 43.8%, while all isolates of *Citrobacter diversus* and *Staphylococcus aureus* could not exhibit heteroresistance to any antibiotic.

Table 3. Heteroresistance profile of bacterial isolates from hospital fomites and hand of healthcare workers

Isolates	Heteroresistance profile	Sources
Escherichia coli	Caz	Table
	Caz	Table
	Amc	Beddings
	amc, imp	Beddings
	fox, caz	Table
	Pn	Sink
	Cn	Table
Pseudomonas aeruginosa	Fox	Table
	imp, caz, fox, e, pef, z, s	Sink
	imp,caz,fox	Staff hand
	amc,cro,imp	Bedding
	caz,amc,cro	Sink
Shigella spp	cro,cpx,s,sxt,e,pef,cn	Door handle
Serratia marcescens	pef,cn,apx,z,am,imp,fox,caz	Table
Providencia rettgeri	fox,pn	Sink
Klebsiella pneumoniae	Sxt	Table

Legend: pef =perfloxacin, cn=gentamycin, S=streptomycin, pn=ampicillin, caz=ceftazidime, amc=amoxicillin-clavulanic acid, e=erythromycin, cro=ceftriaxone, cpx=ciprofloxacin, am=amoxicillin sxt=septrin, fox=cefoxitin

Table 4. Frequency of heteroresistant bacterial isolates

Organisms	No (%)	No (%) Heteroresistant
Escherichia coli	15(20.8)	7(43.8)
Klebsiella pneumoniae	3(4.2)	1(6.3)
Pseudomonas aeruginosa	16(22.2)	5(31.3)
Citrobacter diversus	4(5.6)	0
***Shigella* spp**	2(2.8)	1(6.3)
Providencia rettgeri	4(5.6)	1(6.3)
Serratia marcescens	2(2.8)	1(6.3)
Staphylococcus aureus	26(36.1)	0
Total	72	16(22.2)

4. Discussion

Hemagglutination (HA) and haemolysis of red blood cells are indices of the pathogenic potential of bacterial species. The detection of a wide range of bacterial species that showed hemagglutination and haemolysis of red blood cells in our study area corroborates the report of a previous study[13]. The in-vitro demonstration of hemagglutination is often connected with the activities of hemagglutinins or adhesins which are used for the binding of cellular structures on the surfaces of erythrocyte [14]. Thus, HA-positive isolates in this study can be considered most likely to have p-fimbriae. This is because fimbriated bacteria portrayed hemagglutination properties as previously documented [12, 13].

Fimbriae in bacterial isolates have been identified as appendages mediating the colonization and formation of biofilms on the biotic environment [15] and abiotic surfaces [16]. Fimbriae *in E. coli, P. aeruginosa, and K. pneumoniae* mediate the binding of the bacterial factors to the cellular structures on the epithelial cells of the urinary tract, which is essential in urinary tract infections. In a certain strain of *E. coli* also, fimbriae is essential in the cause of diarrhoea. Therefore, the detection of organisms with hemagglutination-producing traits on hospital fomites and the hands of healthcare workers may contribute to the burden of nosocomial infections involving urinary tract infections, septicemia, diarrhoea, among others in the study area.

Haemolysin production is a virulence factor considered important for some strains of bacteria to overcome host defence mechanisms. Haemolysis of erythrocytes increases the availability of iron in the bacterial environment. This facilitates their survival and invasion potentials in the host tissues. *Escherichia coli* hemolysin was also associated with cytotoxic molecules that has selective activity against neutrophils [17].

The occurrence of biofilm in bacterial isolates connotes the demonstration of a virulence marker that was responsible for most persistent infections [18]. Overall, 31.9% of isolates in this study were detected as biofilm formers. This is lower than reports from previous studies that reported 62.7% [19], 64.3% [20], and 71.8% [21]. More so, the detection of Gram-negative bacteria especially *E. coli, P. aeruginosa, and K. pneumoniae* among biofilm formers in this study was in agreement with previous reports [19, 20]. The majority of biofilm-producing organisms were detected from patients' beddings, healthcare worker's tables, and sinks. Because the sink is usually moist, may facilitate biofilm production.

The high detection of biofilm production among *P. aeruginosa* in this study corroborates the report of an earlier study [22]. According to them, *P. aeruginosa* has the ability to produce biofilm on medical devices and the surfaces of hospital fomites, in addition to medical complications. This may afterward lead to prolonged hospital stays and increased cost of treatment. More so, detection of biofilm-producing organisms particularly *S. aureus K. pneumoniae, E. coli, and P. rettgeri* on these sites, may prolong their longevity on the sites and facilitates their dissemination to susceptible patients in the hospital. As such, infections involving these organisms attached to hospital fomites could constitute a threat to therapy. Studies have shown that more than 80% of microbial infections in humans are biofilm-mediated infections [18, 23].

Organisms that formed or exist in biofilm matrices may harbour chains of benefits. These benefits include but are not limited to easily adherence to *in vivo* or *invitro* surfaces, the decline in growth rate, conferring the ability to evade the host immune systems, bestowing antibiotic resistant phenotype to the bacterial isolate through constraint in the movement of the antibiotic into the cell or the manifestation of chromosomal encoded genes [21, 24]. As such, the biofilm phenotype facilitates the development of multi-drug resistant organisms which often leads to treatment failure [18]. Also, biofilm is responsible for such infections that are lingering, re-occurring, and persistent, leading to a high rate of ill-health and death; thus, posing a serious health crisis [20].

The finding that heteroresistance (HR) can be demonstrated against a variety of antimicrobials and a wide range of bacteria [25, 26] was demonstrated in this study.

Heteroresistance (HR) is not a well-appreciated kind of resistance to the antibiotic; these are because, in HR, a larger population of bacterial species susceptible to a particular antibiotic may contain a marginal population that is resistant to the same antibiotic. This type of resistant phenotype is conspicuously different from the usual and well-documented resistant phenotype where all the cells in the bacterial population are not susceptible to a particular antibiotic [26, 27].

Although not much has been said about the clinical significance of HR [28]. Nonetheless, findings from previous research have shown that though heteroresistant cells constitute marginal of the total susceptible population, they can replicate rapidly; as such, they are prone to facilitate *in vivo* failure in therapy [27, 29] More to this, inappropriate advice, prescription and therapy may be ensured when heteroresistant cells are encountered in hospital laboratories [26]. This is because heteroresistant cells are often neglected or considered susceptible in routine antimicrobial susceptibility testing due to their marginal population.

5. Conclusion

This study elucidates the presence of various caches of virulent markers on bacterial isolates recovered from the hands of healthcare workers and surfaces of hospital fomites in Mubi General Hospital. Each of these markers is unique and functions differently from the other. Infections with bacterial isolates with these traits could be dangerous. We, therefore, advocate speedy decontamination strategies in the hospital to mitigate the spread of these organisms to immunocompromised patients in the hospital, their caregivers, and outpatients.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

Verbal informed consent was obtained from the healthcare workers of the hospital, and consent was sought and obtained from the management of the hospital

Consent for publications

All authors read and approved the final manuscript for publication.

Authors' Contribution

The study was designed by author M.Y.T. who wrote the first draft of the manuscript. Author, J.F. supervised and corrected the draft. Author R.E. and S.E.K. help in sample collection, laboratory procedure, and literature search.

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The authors have embedded all data in the manuscript.

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