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Screening of Hands of Vulnerable Pupils (*Almajiri*) and their Frequently Touched Surfaces for MRSA and ESBL Producing Gram Negative Bacteria

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Abstract

Dissemination of methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum β–lactamases (ESBLs) producing Gram negative bacteria through hands and shared surfaces is widely reported in hospitals and communities. A study was designed to screen hands and frequently touched surfaces of vulnerable pupils for MRSA and ESBL producing *Escherichia coli and Klebsiella pneumoniae*. A total of 400 swabs of hand and frequently touched surfaces of bowl, floor, wall and door knobs shared by *Almajiri* were collected and cultured on MacConkey and Mannitol Salt Agar and incubated at 37 °C for 24 hours. Identification of isolates was done using biochemical tests. Antibiotic susceptibility pattern of the isolates was determined using Clinical Laboratory Standard Institute method. Isolated *S. aureus* were screened for methicillin resistance using cefoxitin disc while *E. coli* and *K. pneumoniae* were screened for ESBL production using double disc synergy test. Out of the 400 samples, 80 (20 %) yielded bacterial growth. A total of 55 (13.75 %) isolates were identified as *S. aureus* followed by 18 *K. pneumoniae* (4.5 %) and 7 *E. coli* (1.75 %). About 24% isolates were recovered from bowl, 3% from door, 8% from floor, 63.75% from hands and 4% from the wall. *S. aureus* was more susceptible to gentamicin 100% and was more resistant to clindamycin 32.72%. *E. coli* was 100% sensitive to gentamycin, cefepime and ceftriaxone and 71.42% was resistant to ampicillin. *K. pneumoniae* was 100% sensitive to ceftriaxone and gentamycin and was more resistant to ampicillin 66.7%. The finding shows that MRSA and ESBL producing bacteria was not detected among the bacterial isolates. It is recommended that convenient sanitary environment should be provided for purpose of *Almajiri* education.

Un Examen des mains d'élèves vulnérables (les *Almajiri*) et de leurs surfaces fréquemment touchées pour le RMSA (la résistance à la méthicilline Staphylococcus aureus) et le BLSE (bêta-lactamases à spectre étendu) produisant des bactéries à Gram négatif

Résumé

La dissémination de Staphylococcus aureus résistant à la méthicilline (le RMSA) et de β–lactamases à spectre étendu (BLSE) produisant des bactéries à Gram négatives par les mains et les surfaces partagées est largement signalée dans les hôpitaux et les communautés. Une étude a été conçue pour examiner les mains et les surfaces fréquemment touchées des pupilles vulnérables à la recherche de RMSA et de BLSE produisant Escherichia coli et Klebsiellapneumoniae. Un total de 400 écouvillons des mains et des surfaces fréquemment touchées de la cuvette, du sol, du mur et des boutons de porte partagés par *Almajiri* ont été rassemblés et cultivés sur MacConkey et Mannitol Salt Agar et incubés à 37°C pendant 24 heures. L'identification des isolats a été effectuée à l'aide de tests biochimiques. Le profil de sensibilité aux antibiotiques des isolats a été déterminé à l'aide de la méthode du 'Clinical Laboratory Standard Institute'. Les S. aureus isolés ont été examiné pour la résistance à la méthicilline à l'aide d'un disque de céfoxitine tandis que E. coli et K. pneumoniae ont été examiné pour la production de BLSE à l'aide d'un test de synergie à double disque. Sur les 400 échantillons, 80 (20 %) ont donné lieu à une croissance bactérienne. Au total, 55 isolats (13,75 %) ont été identifiés comme étant S. aureus suivis de 18 K. pneumoniae (4,5 %) et 7 E. coli (1,75 %). Environ 24 % des isolats ont été récupérés dans la cuvette, 3 % dans la porte, 8 % dans le sol, 63,75 % dans les mains et 4 % dans le mur. S. aureus était plus sensible à la gentamicine à 100 % et plus résistant à la clindamycine à 32,72 %. E. coli était sensible à 100 % à la gentamicine, au céfépime et à la ceftriaxone et 71,42 % était résistant à l'ampicilline. K. pneumoniae était sensible à 100 % à la ceftriaxone et à la gentamicine et était plus résistante à l'ampicilline 66,7 %. La découverte montre que les bactéries productrices de RMSA et de BLSE n'ont pas été détectées parmi les isolats bactériens. Il est recommandé qu'un environnement sanitaire convenable soit fourni aux fins de l'éducation *almajiri*.

Introduction

Emergence of resistance among the most important bacterial pathogens is recognized as a major public health threat affecting humans worldwide (Upreti *et al*., 2018). Resistant organisms have emerged not only in the hospital environment but are now often identified in community settings. The suggests that reservoirs of antibiotics resistance bacteria are present outside the hospital (Munita *et al*., 2016). Antibiotics resistance by such organisms is a major problem faced around the world, of high interest among these antibiotic resistant organisms are Methicillin resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta Lactamases (ESBL) producing *E. coli* and *Klebsiella pneumoniae* which can be easily spread (Jain *et al*., 2008; Jaton *et al*., 2016). The problem of bacteria resistance was earlier overlooked due to the use of wide array of antibiotics from the resistant one to the susceptible type. This problem is a challenge to healthcare workers and other members of the community who are left with no other alternative other than using expensive and toxic antibiotics to treat patience, treatment options are limited in situations where no antibiotic is effective (Frieden, 2010). The monitoring and eradication of MRSA and ESBL producing bacteria from patients, healthcare workers, and their family members is essential to help prevent the continuous spread between hospital and the community (Abu-Rabie, 2010).

Skin hygiene, particularly of hands, is considered to be one of the primary mechanisms in reducing risk of transmission of infectious agents by both the contact and fecal-oral routes (Cobrado *et al*., 2017). Over the decades, bathing, scrubbing, washing traditions and rituals have become established within the healthcare setting.

However, several factors suggest the need for a reassessment of skin hygiene and how it is practiced effectively (Mohammed *et al*., 2015). First, the increasing prevalence of diseases and therapies that compromise immune function means that patients are at higher risk of infections (Larson, 1999). From surfaces, microbial transmission may occur either through direct patient contact or, indirectly, through healthcare personnel hands or gloves (Han, 2015).

Majority of the *Almajiri* schools found in Northern Nigeria lack good sanitary system (Sarkingobir *et al.,* 2019). Consequently, the *Almajiri* children cannot escape being contaminated with microbial population from the environment to fellow *Almajiri* pupils. Hence, the reason for this study to screen frequently touched surfaces and hands of *Almajiri* pupils' for methicillin resistant *Staphylococcus aureus* and extended spectrum beta-lactamases producing *Escherichia coli and Klebsiella pneumoniae*.

Materials and Methods

Study Area and population

A descriptive cross-sectional study was designed and carried out to screen frequently touched surfaces and hands of *Almajiri* pupils for MRSA and ESBL producing *E. coli* and *K. pneumoniae*.

Samples of hand and touch surfaces of *Almajiri* were collected from five *Almajiri* schools situated in five Local Governments Areas (Gwale, Tarauni, Kano Municipal, Kumbotso and Nassarawa) of Kano, Nigeria from November to December, 2019. Samples were collected from *Almajiri* pupils that live in overcrowded and poorly ventilated rooms situated very close to either gutters, open defecation sites or dump sites.

Ethical clearance

Before the study commenced, ethical clearance was obtained from Health Ethics Committee of the Kano State Ministry of Health. Ethical approval (Ref: MOH/Off/797/T.I/1303) was given in favour of the conduct of this research. Participants' consents were also obtained prior to the commencement of the study.

Inclusion and Exclusion Criteria

Male *Almajiri* pupils aged between 5 and 10 years, and matched apparently healthy individuals during sample collection period were included in the study. However, *Almajiri* pupils who were not willing to fill the informed consent form were all excluded from the study.

Sample Collection

Total of 400 swab samples of hand and frequently touched surfaces of bowl, floor, wall and door knobs were collected from the selected schools. Sterile cotton swab sticks pre-moistened in sterile normal saline were used to swab the touch surfaces and inter-digital spaces of both left and right hands of the pupils as described by Cetin *et al*., (2006). The samples were properly labelled and dispatched to the Microbiology Laboratory immediately. Information such as age, washing of hand before and after eating and also after using the toilet was obtained from each *Almajiri* pupils.

Isolation and Identification of Bacterial from samples

The samples were inoculated on Nutrient agar, Mannitol salt agar and MacConkey agar followed by incubation at 37ºC for 24hours. Bacteria that grew on the media were identified by first observing their growth morphology on various culture media and then Gram stained to observe their shape and gram reaction. Identity of the bacteria was confirmed by series of biochemical tests (Catalase test, Coagulase test, Indole test, Citrate utilization, Urease production and triple sugar iron) (Cheesbrough, 2006).

Antimicrobial Susceptibility Pattern of Bacteria Isolates

Disc diffusion method of CLSI 2019 was used for determining the antimicrobial susceptibility of the isolates on Muller Hilton Agar (MHA). The MHA was evenly seeded with MacFarland standard of the test organisms using a swap stick (Abubakar, 2018). Discs of antibiotic gentamycin (10 µg), tetracycline (30 µg), clindamycin (5 µg), penicillin (30 µg), levofloxacin (30 µg) and erythromycin (10 µg) were tested against *S. aureus* whereas antibiotics ampicillin (10 µg), ceftriaxone (30 µg), gentamycin (10 µg), ciprofloxacin (5 µg), cefepime (30 µg), and tetracycline (30 µg) were tested against *E. coli* and *K. pneumoniae*. After 24h of incubation period at 37ºC, the zones of inhibition were measured in mm, then the results were analyzed according to the guidelines issued by the Clinical Laboratory Standard Institute (CLSI, 2019).

Screening *S. aureus* for Methicillin Resistance

Detection of methicillin resistance among *Staphylococcus aureus* isolates was done using Cefoxitin disc (30 µg) diffusion test and results was interpreted according to CLSI guidelines (CLSI, 2019). Any *S. aureus* that is resistant to cefoxitin (that produce zone of inhibition ≤ 21mm) was considered MRSA.

Detection of ESBL production in *E. coli* and *K. pneumoniae*

Screening *E. coli* and *K. pneumoniae* for ESBL production was performed using CLSI's Double disc synergy test. In the test, a disc of augmentin (20 µg amoxicillin and 10 µg clavulanic acid) was placed at the centre of each inoculated MHA plate. Cefotaxime (30 µg) and ceftazidime (30 µg) discs were then placed 20 mm (centre to centre) from the augmentin disc and incubated at 37ºC overnight. Enhancement of zone of inhibition of any cephalosporin beta-lactam antibiotic disc (i.e. Cefotaxime and ceftazidime) caused by the synergy with clavulanate in the amoxicillin/ clavulanate disc was taken as ESBL production (CLSI, 2019).

Data Analysis

The generated data in this study were analysed using statistical analysis package software (SPSS version 20.0). The statistical tool used was chi-square. The confidence level 95% was used in this study. $P < 0.05$ was considered significant.

Result

Result of the present study revealed that, of the 400 swab samples collected and subjected to bacteriological analysis, 80 (20%) isolates comprising *Staphylococcus aureus* 55 (13.75%), *Klebsiella pneumoniae* 18 (4.50%) and *Escherichia coli* 7 (1.75%) were isolated (Table 1).

Table 2 shows the overall *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* isolated from *Almajiri* pupils based on location of the *Almajiri* school. The result shows that 20 bacteria out of the 80 were isolated from schools situated in Nassarawa LG while schools in Kumbotso had the lowest number of bacteria 11(21.2%).

The distribution of bacteria isolated from hands and frequently touched surfaces of *Almajiri* Pupils based on age is presented in Table 3. *Almajiri* pupils of age 5 and 10 showed lower number of *E. coli*, *K. pneumoniae* and *S. aureus* when compared with those in the age range of 6 to 9. The result also shows that there is no significant difference between ages of the *Almajiri* pupils and the bacterial isolates ($P > 0.05$).

Table 1: Prevalence of Bacterial isolates in the study samples

Bacterial isolates (*n* = 400)	**Occurrence (%)**
Escherichia coli	7 (1.75)
Klebsiella pneumoniae	18 (4.50)
Staphylococcus aureus	55 (13.75)
Total	80 (20)

Table 2: Distribution of Bacterial Isolates from Swab Samples based on Location

Location	**No. of samples examined (%)**	**No. of samples that yield bacteria growth (%)**
Gwale	80 (20.00)	15(3.75)
Tarauni	81 (20.25)	14 (3.50)
Kano Municipal	64 (16.00)	16 (4.00)
Kumbotso	52 (13.00)	11 (2.75)
Nassarawa	83 (20.75)	20 (5.00)
Control	40 (10.00)	4 (1.00)
Total	400 (100)	80 (20)

$\chi 2 = 10.171$, $P = 0.070$

Table 3: Distribution of Bacterial Isolates from Swab Samples based on Age

Age	**No. Examined (%)**	**No. of Growth (%)**	***E. coli* (%)**	***K. pneumoniae* (%)**	***S. aureus* (%)**
5	19	4 (7.84)	0(0)	0(0)	4(6.97)
6	48	17 (33.33)	1(50.00)	1(16.66)	15(34.88)
7	62	13 (25.49)	0(0.00)	0(0.00)	13(30.23)
8	30	8 (15.68)	0(0.00)	2(33.33)	6(13.95)
9	40	7 (13.72)	0(0.00)	2(33.33)	5(11.62)
10	42	3 (5.88)	1(50.00)	1(16.66)	1(2.32)
Total	241	51 (21.16)	2(3.92)	6(11.76)	43(84.31)

$\chi 2 = 16.829$, $P = 0.0048$

The frequently touched surfaces of bowl, door, floor and walls of shared rooms of *Almajiri* pupils yielded varying amount of bacteria and the result is shown in Table 4. Bacteria isolated from hands were the most predominant positive isolates, 51(63.75%), followed by isolates from bowl 18 (22.5%), floor 6(7.5%), wall 3(3.75%) and door knob 2(2.5%). There was significant difference ($P < 0.05$) between the distribution of bacterial isolates and the surfaces where they were isolated.

Table 5 shows that there is no significant ($P = 0.5571$) difference between *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and hand washing before eating. Washing hands after eating and washing hand after visiting toilet does not have significant difference with the isolated bacteria ($P > 0.05$).

Table 4: Distribution of Bacterial Isolates from Hands and frequently touched surfaces

Surfaces	**No. of positive isolates**	**Bacterial isolates**			***P-value***
		E. coli (%)	*K. pneumoniae* (%)	*S. aureus* (%)	
Bowl	18(22.5%)	4(22.22)	10(55.55)	4(22.22)	0.022
Door	2(2.5%)	0(0.00)	0(0.00)	2(100)	
Floor	6(7.5%)	1(16.66)	2(33.33)	3(50.00)	
Hand	51(63.75%)	2(3.92)	6(11.76)	43(84.31)	
Wall	3(3.75%)	0(0.00)	0(0.00)	3(100)	
Total	80 (100)	7(8.75)	18(22.5)	55(68.75)	

Table 5: Distribution of bacterial isolates according to possible risk factors

Variable	Total No. Examined	No. of positive	*E. coli* (%)	*K. pneumoniae* (%)	*S. aureus* (%)	*P*-value
WHBE						
Yes	56	18	0	2	16	0.5571
No	185	33	2	4	27	
WHAE						
Yes	91	23	1	1	21	0.3294
No	150	28	1	5	22	
WHAVT						
Yes	28	19	0	3	16	0.4483
No	218	32	2	3	27	
Bathing						
Everyday	0	0	0	0	0	
Once a week	241	51	2	6	43	
Wearing clean clothes						
Yes	0	0	0	0	0	
No	241	51	2	6	43	

Risk factors with $P < 0.05$ are considered significant.
Abbreviations: WHBE- washing of hands before eating, WHAE- Washing of hands after eating, WHAVT- Washing of hands after visiting toilet.

Table 6 shows the antibiotics susceptibility profile of *Staphylococcus aureus* isolated from *Almajiri* pupils' hands and frequently touched surfaces. *Staphylococcus aureus* was more sensitive to gentamycin (100%) while exhibiting the highest resistance to clindamycin (32.72%). The antibiotic susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* bacterial isolates showed that the Gram-negative bacteria were 100% sensitive to ceftriaxone and gentamycin, while the highest resistance of the isolates was observed against ampicillin (Table 7).

The present result shows the percentage prevalence of ESBLs producing *Escherichia coli* and *Klebsiella pneumoniae* (Table 8). *Escherichia coli* and *Klebsiella pneumoniae* are not ESBL producers. In addition, *Staphylococcus aureus* isolated from this study are not resistant to methicillin, meaning they are methicillin susceptible.

Table 6: Antibiotic susceptibility profile of S. *aureus* (*n* = 55) isolated from *Almajiri* pupils' hands and frequently touched surfaces

Antibiotics	Sensitive (%)	Resistant (%)
Clindamycin	37(67.27)	18(32.72)
Penicillin	41(74.54)	14(25.45)
Levofloxacin	53(96.36)	2(3.63)
Erythromycin	40(72.72)	15(27.27)
Gentamycin	55(100)	0(0.00)
Tetracycline	48(87.27)	7(12.72)

Table 7: Antibiotics susceptibility profile of *E. coli* and K. *pneumoniae* isolated from *Almajiri* pupils' hands and frequently touched surfaces

Antibiotics	***Escherichia coli* (n=7)**		***Klebsiella pneumoniae* (n=18)**	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ceftriaxone	7(100)	0(0.00)	18(100)	0(0.00)
Ampicillin	2(28.57)	5(71.42)	6(33.3)	12(66.7)
Gentamycin	7(100)	0(0.00)	18(100)	0(0.00)
Ciprofloxacin	3(42.85)	4(57.14)	7(38.9)	11(61.1)
Tetracycline	3(42.85)	4(57.14)	9(50)	9(50.00)
Cefepime	7(100)	0(0.00)	15(83.33)	3(16.6)

Table 8: Prevalence of ESBL Producing *E. coli* and K. *pueumoniae*

Bacteria	No of Isolates	ESBL
Escherichia coli	7	0(0)
Klebsiella pueumoniae	18	0(0)
Total	25	0

Discussion

According to Larson (1999), skin hygiene particularly of hands, is considered to be one of the primary mechanisms in reducing risk of transmission of infectious agent by both direct contact and fecal-oral routes. In this study, 400 samples, 241 from hands, 12 from door handles, 30 from floor, 35 from wall and 82 from bowls were collected and screened for MRSA and ESBLs producing bacterial isolates. *S. aureus* had the highest occurrence (55, 13.75%), followed by *K. pneumoniae* (18, 4.50%) and *E. coli* (7, 1.75%). Findings on the high prevalence of *S. aureus* in this study are consistent with previous studies conducted by Kumari *et al*., (2015) and Upreti *et al*. (2018). Studies revealed *S. aureus* to be a normal flora of the skin (Maji *et al*., 2018), which probably explains its high prevalence in the present study. Similarly, the limited ability of *E. coli* to survive on hands and other environmental surfaces explains the lower occurrence of the bacterial isolate in the current study. According to Scott *et al.* (2008), *E. coli* can survive only for a short time on hands and other environmental surfaces.

The antimicrobial sensitivity of *S. aureus* indicated that penicillin, levofloxacin, erythromycin, gentamycin and tetracycline are potent antibiotics against *S. aureus*. The result further showed that gentamycin was more effective against *S. aureus*. High resistance to clindamycin (32.72%) was recorded in this study, indicating that this antibiotic might not be very effective in the treatment of *S. aureus* infection. This study showed high sensitivity rate as compared to other studies (Upreti *et al*., 2018). The antimicrobial susceptibility pattern of *E. coli* and *K. pneumoniae* indicated that Cefepime, gentamycin and ceftriaxone are potent antibiotics against *E. coli* and *K. pneumoniae*. Ampicillin showed high rate of resistance to *E. coli* and *K. pneumoniae*, indicating that these antibiotics are not effective for the treatment of *E. coli* and *K. pneumoniae* infections. *K. pneumoniae* was most sensitive to gentamycin (100%) and ceftriaxone (100%) and 66.7% and 61.1% of *K. pneumoniae* isolates were equally resistant to ampicillin and ciprofloxacin respectively. In a study reported by Mama *et al*. (2014), 35.7% of *K. pneumoniae* were resistant to ciprofloxacin, while Rajput *et al*. (2008) reported 45.5% were resistant to ciprofloxacin by *K. pneumoniae*.

The widespread use of broad spectrum β-lactam antibiotics has led to a marked increase in the incidence of ESBL in Gram negative organisms (Rawat and Nair, 2010). This study reveals that no MRSA and ESBLs producing bacteria was isolated from this study. This could be due to lack of exposure to antibacterial substances or herd immunity. The present finding corresponded with the study of Peter *et al*. (2017) who found 0%

prevalence of MRSA and ESBL from Multidrug-Resistant organisms in their study subjects. Contrary to the current finding, Oyetunji *et al.* (2012) isolated 63.3% MRSA from healthy community individuals from Jos South, Nigeria. In addition, Muge *et al*. (2007) found 5.6% prevalence of MRSA from healthy *Preschool* children. However, Javid and Rajesh (2013) found 9 MRSA out of 100 *S. aureus* isolated from a healthy community. This research agrees with that of Suen *et al*., (2019), who observed and reported no MRSA on surfaces but one strain of Methicillin resistant *Staphylococcus* epidermis (MRSE) and Methicillin resistant *Staphylococcus saprophyticus* (MRSS) out of 3, 24 and 4 isolates respectively. According to Scott (2008), having a child or being a healthcare worker is not a significant predictor of whether MRSA would be found in the school or not. Even though ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* have been reported in environmental sample (Okesola and Fowotade, 2012), it is not reported in this study. This may be due to the fact that ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* are mostly associated with clinical specimens (Upreti *et al*., 2018). However, even though ESBL and MRSA was not isolated in this study, the bacteria *S. aureus*, *E. coli* and *K. pneumoniae* isolated from this work were found to be resistant to some antibiotics. The reason for the high-level resistance to antibiotics in *Almajiri* pupils may not necessarily reflect exposure in individual pupils, but exposure from their contacts; indicating that community level exposure to antibiotics may play a greater role in the dissemination of resistant bacteria than individual exposure in the pupils (Ashley *et al.,* 2016).

Conclusion

In this study, the most common isolate from the hands and frequently touched surfaces of *Almajiri* pupils was *Staphylococcus aureus* (13.75%) followed by *Klebsiella pneumoniae* (4.5%) and *Escherichia coli* 1.75%). The positive bacterial isolated from this study showed low resistant to the antibiotics. There was no strain of MRSA and ESBLs producing bacteria isolate was isolated. Hence, the *Almajiri* pupils could be considered potential carriers of *S. aureus*, *E. coli and Klebsiella pneumoniae.* Implementation of infection control measures, restricting the use of broad-spectrum antibiotics, rotation of antibiotics and rationalizing the use of antibiotics can decrease antibiotics resistance. In addition, adequate sanitary care of the environment should be enforced to curtail the spread of antibiotics resistance.

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