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Microbial Contents and Antibiotics Susceptibilities from Hand Washing Stations during COVID-19 Pandemic in Lagos, Nigeria

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Abstract

The COVID-19 pandemic put every government in the globe on red alert; safety protocol and long-standing infection prevention and control (IPC) measures such as hand and oral hygiene, social/ physical distancing, use of face mask were rigorously enforced. This study evaluated microbial (bacterial, fungal) contents of rinsate, bowl water, water from tap, and the neighborhood drainage in selected hand washing stations in Agege, Alimosho, Mainland, Island and Shomolu local government areas (LGAs) in Lagos, Nigeria. The identities of bacterial isolates were confirmed with 16S rRNA sequencing while the fungi were identified by colonial appearances. The antibiotics susceptibility testing (AST) of the bacteria against structurally unrelated antibiotics was performed and interpreted according to standard guidelines. Sixty-seven (67) bacterial and fifty-one (51) fungal isolates were recovered from mainly water bucket with tap and rinsates across all the LGAs. There were marked microbial loads (some too numerous to count at 10^{-3} dilution) across Alimosho, Lagos Island and Mainland. Forty (40) representative bacterial isolates were selected for 16S rRNA sequencing. The occurrence of microbial isolates in the samples was at varying degrees; *Klebsiella* spp. (37.5%), *Bacillus* spp. (32.5%), *Enterobacter* spp. (17.5%), were the predominant bacteria while *Aspergillus* spp. (46.2%), yeasts (34.6%), *Sporothrix schenckii* (11.5%) and *Penicillium* spp. (7.7%) constituted the fungal isolates. The biological weapon, *B. anthracis* was recovered from a water bucket with tap in Alimosho. The antibiotics susceptibility testing of the bacteria showed high degrees

of resistance profile; 45 (69.2%) to ampicillin, 41.5% amoxicillin/clavulanate while 47.6% were resistant to two (2) or more antibiotics. This study demonstrated high microbial load during the pandemic at the study LGAs, presence of environmental commensals reputed for debilitating opportunistic infections in man and risk of passing heavy load of these potential pathogens to the public.

Keywords

Microbial, Antibiotics, Handwashing, COVID-19, Rinsates

1. Introduction

The global hand washing day is celebrated on October 15, last year had the theme "hand hygiene for all" with its importance underscored in the prevention of COVID-19 pandemic. Hand washing is a sure way of preventing communicable diseases such as cholera, common cold, some foodborne diseases/gastrointestinal norovirus, Lassa fever and acute respiratory diseases [1]-[8]. This is more so because human hands harbor microorganisms that may both be transient or residents (normal flora) and constitute primary mode of transmission of many infectious agents [9].

The removal of transient and/or resident flora from the skin is dependent on some factors (microbial load, efficacy of hand washing or for how long it was done, types of soap, organic matter), while certain spp. persist even with the use of antimicrobial soap with water, others are easily washed off [10].

In the era of COVID-19 pandemic, with more emphasis on hand hygiene, more people tended to practice hand washing using various types/brands of antiseptics/disinfectant soaps and alcohol based hand rubs. This will wash off more of microorganisms from the hand and one way or the other find their way into community drainage system. These colonizing microorganisms which might not have been totally killed by the hand washing agents will constitute health hazards to the environment; this is because their removal from soiled hands and resultant killing are dependent on; microbial load, organic contents, hand washing time, soap used. It is also important to note that persistent washing by many people will increase the probability of reducing the killing ability of the soap in the rinsates.

Provision of improvised buckets with tap or a bowl of water for hand washing was commonplace in the era of COVID-19 pandemic in shopping malls, institutions, market place, healthcare facilities, schools, et cetera.

The practice of not changing rinse water in the bowl frequently as a result of lack of running tap water could increase the rate of exchange of microbes between various users and this constitutes ineffective hand washing [11] [12].

This study, therefore, was designed to evaluate the microbial (bacterial, fungal) contents and antibiotics susceptibilities in water used in hand washing and

in drainage systems within the neighborhood of selected stations in Lagos State.

This will help to determine the health hazard posed by the ubiquitous, sub-standard hand washing stations made during the pandemic.

2. Materials and Methods

2.1. Sites

Selected hand washing stations in five LGAs in Lagos, Nigeria namely Agege (Ag), Alimosho (Al), Island (Is), Mainland (Mn), and Shomolu (Sh). These stations cut across shopping malls, churches, schools, health care facilities (hospitals, laboratories), pharmacy/cosmetic stores, eateries/restaurants, hotel/bar, factories, gas/fuel stations, tax office and residential buildings

2.2. Ethical/Social Consideration

The study did not involve any human participants. The approval (IRB/20/097) was obtained at the institutional review board of the Nigerian Institute of Medical Research (NIMR-IRB).

The permission to conduct the study was granted by the Lagos State Primary Health Care Board (LS/PHCB/MS/1128/VOLVIII/093) while community entry was facilitated by the respective Medical Health Officer (MHO) in each local government.

2.3. Sampling/Microbiology

The rinsates, bowl water, taps and drainage water samples were collected in screw capped containers following standard aseptic procedures between 5th March 2021 to 6th May 2021 (9 weeks). The five LGAs were selected purposively while the water samples were collected by convenience and also based on the set-up at the stations.

They were transported (in ice packs) to the laboratory for processing; ten (10) fold serial dilutions of the samples were done in duplicates while 100 µl of the diluted sample was inoculated in petri dish for pour plating on bacteriological media (MacConkey, Mueller Hinton agar (MHA)) and spread plating on Sabouraud dextrose agar (SDA) for fungi following all standard procedures. Incubation was done in air at 37˚C for 24 hr. for bacteria and room temperature for up to a week for fungi.

The microbial counting was done and recorded as from no growth to too numerous to count (TNTC).

2.4. Antibiotics Susceptibility Testing (AST)

This was performed by agar disc diffusion methods following the guidelines of Clinical and Laboratory Standard Institute (CLSI). Briefly, suspension of the distinct colonies (adjusted to 0.5 McFarland turbidity) was made in sterile physiological saline and with the aid of sterile swab stick, lawns of the suspension were made on the surface of sterile MHA plates, before placing the antibiotic discs.

This was incubated in air at 37˚C for 24 hr. after which the inhibition zone was measured in millimeter (mm). The antibiotics were selected based on the Gram stain reaction of the bacterial isolates.

2.5. Identification

The combination of colonial morphology, biochemical and molecular assays were performed on distinct (single, pure) colonies.

Forty bacterial isolates were selected and confirmed with 16S rRNA sequencing using primers targeting the hyper variable regions V5-V6-V7 to amplify the target DNA after which sequencing was carried out on the amplicons. The representative bacteria were selected from isolates showing similar colonial and biochemical characteristics. All fungal isolates were identified with colonial morphology.

DNA Extraction and Polymerase Chain Reaction (PCR)

The extraction of DNA from the bacterial isolates was performed using in-house NIMR-BIOTECH kit and quantitation done by qubit fluorometer.

The PCR was performed in 20 µl reaction comprising the primer pair (799f-AACACGGATTAGATACCCG, 1193r-ACGTCATCCCCACCTTCC), ready to load master mix (SolisBiodyne, Estonia), template DNA and distilled water. The amplification was done on thermocycler at specific cycling parameters as follows; initial denaturation at 94˚C for 3 mins, 35 cycles of denaturation at 94˚C for 1 min, annealing at 53˚C for 1 min and elongation at 72˚C for 1 min. The final elongation was carried out at 72˚C for 10 mins before holding the reaction at 4˚C till removal from the cycler.

The amplicons were later resolved alongside a 100 bp DNA ladder (ThermoFisher Scientific, USA) on 1.5% agarose gel run at 100V for 1hr and viewed under UV trans-illuminator.

All amplicons corresponding to the expected gene size (394 bp) were sent out for commercial Sanger sequencing and the data subjected to basic local alignment search tool (BLAST) algorithm on National Center for Biotechnology Information (NCBI) GenBank. All the sequence data (16S rRNA genes) were submitted to the GenBank and accession numbers allocated (Table 1).

3. Results

Sixty-seven (67) bacteria and fifty-one (51) fungal isolates were recovered from different samples collected across the five (5) LGAs while the fungi were identified with phenotypic characteristics, forty representative bacterial isolates were selected for 16S rRNA sequencing based on their different colonial appearances and Gram stain reaction. Most are environmental isolates while some are reputed for opportunistic infections in man.

The confirmed bacterial isolates and frequencies are as follows; *Klebsiella pneumoniae*, 32.5%, *Enterobacter* spp., 17.5%, *Bacillus* spp. 32.5%, *K. aerogenes*, 5.0%, others 12.5%.

Table 1. The identified bacterial species across the LGAs.

SN	Location/LGA	Sample	Bacteria	Accession no
1	Ag	Bw	*Klebsiella pneumoniae*	OK316829
2	,,	Bw	*Enterobacter cloacae*	OK316838
3	,,	Bw	*Bacillus subtilis*	OK316850
4	,,	Bw	*B. subtilis*	OK316851
5	,,	Dr	*B. subtilis*	OK316854
6	,,	Rs	*B. thuringiensis*	OK316855
7	,,	Rs	*B. subtilis*	OK316856
8		Rs	*B. thuringiensis*	OK316859
9	,,	Rs	*B. amyloliquefaciens*	OK316860
10	Al	Rs	*K. pneuminiae*	OK316825
11	,,	Rs	*B. aerius*	OK316826
12	,,	Rs	*E. ludwigii*	OK316827
13	,,	Rs	*K. pneumoniae*	OK316841
14	,,	Bw	*K. pneumoniae*	OK316832
15	,,	Bw	*Cedecea neteri*	OK316834
16	,,	Bw	*B. megaterium*	OK316839
17	,,	Bw	*K. pneumoniae*	OK316845
18	,,	Bw	*B. anthracis*	OK316847
19	,,	Bw	*Uncultured rastonia*	OK316848
20	,,	Bw	*B. amyloliquefaciens*	OK316857
21	,,	Bw	*B. thuringiensis*	OK316858
22	Is	Rs	*E. cloacae*	OK316822
23	Mn	Bw	*K. pneumoniae*	OK316821
24	Mn	Bw	*Stenotrophomonas maltophillia*	OK316824
25	Mn	Bw	*Kluyvera cryocrescens*	OK316833
26	Mn	Bw	*Klebsiella aerogenes*	OK316837
27	Mn	Bw	*K. pneumoniae*	OK316842
28	Mn	Bw	*K. pneumoniae*	OK316843
29	Mn	Bw	*K. pneumoniae*	OK316846
30	Mn	Rs	*K. pneumoniae*	OK316823
31	Mn	Rs	*K. pneumoniae*	OK316828
32	Mn	Rs	*Atlantibacter hermannii*	OK316830
33	Mn	Rs	*K. aerogenes*	OK316831
34	Mn	Rs	*E. ludwigii*	OK316835
35	Mn	Rs	*Enterobacter soli*	OK316836
36	Mn	Rs	*K. pneumoniae*	OK316840
37	Mn	Rs	*E. cloacae*	OK316844
38	Mn	Rs	*K. pneumoniae*	OK316849
39	Sh	Rs	*B. subtilis*	OK316852
40	Sh	bw	*E. cloacae*	OK316853

The agarose gel electrophoresis picture showed the 16S rRNA amplicons at the expected size of 394 bp (Figure 1). The 16S rRNA sequence data were submitted to the NCBI, GenBank and accession numbers allocated (OK316821-OK316860) Table 1.

Many of the fungal isolates were moulds (*Aspergillus* spp 46.2%, Yeasts 34.6%, *Sporothrix schenckii* 11.5%, *Penicillium* spp. 7.7%) and were recovered from water bucket with tap (bw) and the rinsates (rs) which constituted the largest number of samples collected (Table 2). Most of the organisms isolated from the rinsates were also present in the water bucket with tap.

The bacterial count (bc) in the 5 LGAs varied greatly, with Alimosho rs having between non to too numerous to count (TNTC). The highest microbial load was found in bw and rs at Alimosho, Lagos Island and Mainland LGAs while the tap water (tp) used for hand washing did not yield any significant microbial load ($p < 0.05$). Most of the fungal isolates (fc) were recovered from Island and Mainland (Table 3).

The antibiotics susceptibilities result indicates a range for 2 or more bacterial spp. of same genus. For instance, *Klebsiella* spp. include *K. pneumoniae* and *K. aerogenes*. Forty-five (69.2%) of the isolates were resistant to ampicillin while 41.5% were resistant to augmentin and 47.6% multidrug resistant (Table 4).

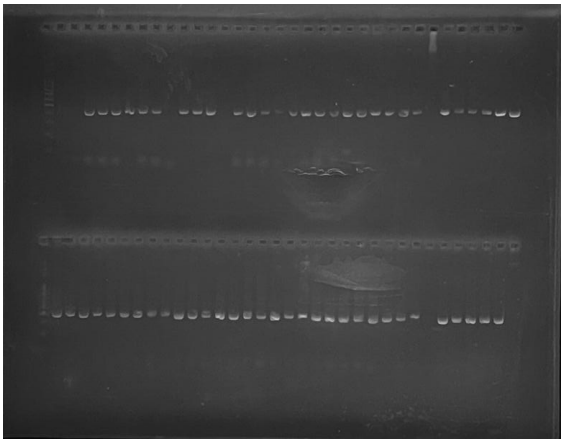

Figure 1. Gel picture of 16S rRNA genes. L-R-Lane 1 (upper/lower): 100 bp DNA ladder, lanes 2-35: amplicons.

Table 2. Fungal isolates across the LGAs.

SN	LGA	Sample	Probable identification
1	Ag	bw	*Aspergillus* spp., yeast
		rs	*Aspergillus* spp., yeast
2	Al	bw	*Aspergillus niger*, *Aspergillus flavus*, *Sporothrix schenckii*, yeast
		rs	*A. niger*, *A. candidum*, *Penicillium* sp., *S. schenckii*, yeast
		dr	Yeast
		tp	*A. niger*, yeast
3	Mn	bw	*A. niger*, *A. flavus*, *Penicilium* sp., yeast
		rs	*A. flavus*, *A. niger*, *S. schenckii*, yeast
4	Is	Bw	*Aspergillus* spp, yeast

Table 3. Total microbial load of the samples per locations (LGAs).

Sample	LGA/microbial load (cfu/ml)				
	Ag (n = 25)	Al (n = 31)	Is (n = 3)	Mn (n = 32)	Sh (n = 10)
Bw	[bc]0-400 [fc]1-50	[bc]0-450 [fc]10-52	[bc]300 [fc]TNTC	[bc]0-50 [fc]1-153	-
Dr	[bc]150	0 0-1	[bc]250 [fc]300	-	-
Rs	[bc]1-400 [fc]1-5	[bc]0-TNTC [fc]12-57	[bc]300 [fc]450	[bc]0-150 [fc]1-TNTC	[bc]1-400
Tp	-	[bc]0-1 [fc]9-35	-	-	-

Ag-Agege, Al-Alimosho, Is-Island, Mn-Mainland, Sh-Shomolu, bw-water bucket with tap, dr-drainage, rs-rinsates, tp-tap. TNTC-Too numerous to count, bcbacterial count, fcfungal count, no samples.

Table 4. Antibiotics susceptibilities of the isolated bacteria.

Bacteria	Antibiotics/zones of inhibition (mm)									
	AMP	CAZ	IMP	GEN	AZI	AUG	CPR	CEF	TET	CHL
Klebsiella spp	0 - 15	0 - 18	0 - 30	0 - 18	0 - 11	15 - 27	18 - 26	18 - 22	0 - 18	16 - 25
Bacillus spp.	10 - 25	15 - 25	18 - 35	15 - 25	18 - 28	18 - 26	0 - 14	0 - 12	0 - 13	12 - 19
Enterococcus spp.	0 - 16	18 - 28	20 - 25	0 - 12	0 - 28	20 - 27	12 - 22	18 - 28	0 - 18	20 - 26
Enterobacter spp.	12 - 23	12 - 20	18 - 32	0 - 16	0 - 20	18 - 27	20 - 22	10 - 18	10 - 16	18 - 21
C. neteri	0	17	28	0	18	28	20	25	0	0
S. maltophillia	0	18	28	11	18	0	25	20	0	0
K. cryocrescens	0	15	29	25	18	21	28	22	0	23
A. hermannii	10	0	32	10	26	28	27	15	0	20

AMP-Ampicillin, CAZ-Ceftazidime, IMP-Imipenem, GEN-Gentamicin, AZI-Azithromycin, AUG-Augmentin, CPR-Ciprofloxacin, CEF-Cefotaxime, TET-Tetracycline, CHL-Chloramphenicol.

4. Discussion

Handwashing, when performed effectively and frequently, has been demonstrated to reduce/eliminate carriage of pathogenic microorganisms and thus reduction in infections/illnesses whether in the community, hospitals settings or farmworkers with soiled hands [11] [13] [14] [15] [16] [17].

The awareness and rush for handwashing were intensified during the COVID-19 pandemic especially in Lagos, being the epicenter in Nigeria. It was not uncommon to see handwashing facilities of various designs in public institutions, where people were made to use before access. This was corroborated by the report of Moore *et al.* 2020 [18] that hand hygiene performance initially increased at the outset of the COVID-19 crisis although this was not sustained. It should be noted however that the investigation mentioned above (Moore *et al.* 2020) [18] was conducted in healthcare settings. Some studies investigated multiple handwashing per day (up to 10 times) and found out this significantly reduced the risk of acquiring seasonal coronavirus infections [19] [20].

Our study conducted in five LGAs in Lagos State had the main objective of investigating bacterial/fungal morphotypes in the rinsates, bowl water, tap, and drainages in selected handwashing stations. This was motivated by paucity of data in Nigeria at the time and the potential hazard the "pressure" can cause to public health.

The work of Tetteh-Quarcoo *et al.* 2016 [21] in preschool children within Accra, Ghana, reported eight different bacterial and fungal isolates which were quite different from our findings may be due to the different situations (as ours was in the midst of pandemic) and the target population. Also, our bacterial isolates were confirmed with 16S rRNA sequencing while theirs phenotypic.

Most of the bacterial organisms were isolated from water bucket with tap (bw) and rinsates (rs) with particularly loads too numerous to count (TNTC). The LGA where this was pronounced is the most populous and even the largest in size.

The bacterial isolates are mostly environmental/enteric commensals with abilities as opportunistic infectious agents in hospitalized or immunocompromised patients. Some have been implicated in debilitating infections with multidrug resistance [22] [23] [24] [25]. For instance, carbapenemase producing *Atlantibacter hermannii* was recently isolated from clinical sample [26]. *Klebsiella* spp. accounted for the highest occurrence in the hand washing stations and this is worrisome, as they are reportedly opportunistic infectious agents especially the *K. pneumoniae*. Although they colonize the mucosal surfaces without causing causing infections, there have been various reports of abilities to cause life threatening infections such as pneumonia, sepsis and other systemic infections as a result of their dissemination to tissues and their abilities to evade host immunity [27] [28]. The high case fatality of between 18% - 49% by especially the multi drug resistant isolates has been reported [29] [30]. In this study, all the isolates of *K. pneumoniae* were multi drug resistant including their resistance to imipenem. This is in tandem with various reports of the emergence of carbapenemase carrying *Klebsiella* spp. especially *K. pneumoniae* [31] [32] [33]. Most of the *Bacillus* spp. recovered from the samples in this study belong to the "cereus" group which has pathogenic potentials while *B. anthracis* causes anthrax, others either cause food poisoning or localized wound/eye infections and systemic disease.

The *B. anthracis* isolated in this study has 100% identity with the one in the GenBank; there is need to perform PCR detection for the important toxins (*pag*A, *cap*) of the anthrax bacillus to confirm their toxigenicity. This isolation is not surprising as the settlements in this LGA have predominantly popular markets for grazing animals living closely with the humans. There is need to urgently put surveillance systems in place to prevent possible outbreak of this important biological weapon. On the other hand, *B. subtilis* is a well reported safe and reliable human and animal probiotic, also with antimicrobial properties [34] while *B. aerius* is reputable for its biotechnological activities [35] [36].

The fungal isolates in this study, also mainly from bw and rs were identified

based on colonial morphology and most are *Aspergillus* spp. while the dimorphic fungus, *S. schenckii* the causative agent of sporotrichosis (a sub-acute and chronic infection) was also isolated. The recent study suggested the association of this organism with cutaneous mast cells (MCs) with marked increase in TNF and IL-6 in skin lesions and in the serum [37].

This study was not able to collect samples from all the LGAs in Lagos due to restriction in movement. The fungal identification using phenotypic characteristics was necessitated by paucity of funds on one hand (as the funding support was quite lean) and difficulty (flight/shipment) in procuring some molecular consumables during the pandemic on the other.

5. Conclusion

Although handwashing has been variously demonstrated to prevent transmission of pathogenic microbes, the pressure during COVID-19 pandemic could cause a spike in pathogenic microorganisms which poses hazard to public health. In this study, the microbial load was heavy in the water bucket and rinsates and isolation of anthrax bacilli in particular is a red flag that should be treated seriously.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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