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Original article

Bacterial contaminants of new disposable ready-to-use plastic cups sold within Gombe Metropolis, Gombe State, Nigeria

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ABSTRACT

Background: Disposable cups are usually used for drinking without any form of pre-cleaning, this has the potential to expose individuals to pathogenic microorganisms, and unfortunately this issue has not been investigated in Gombe metropolis, hence, this study aimed to investigate the bacteriological aspect of the problem and to reduce the existing knowledge gap regarding this problem. **Methods:** Thirty samples were collected using simple random sampling from two different markets, then swab sticks pre-moistened with peptone water were used to swab the inner portions of the cups, put in sterile distilled water, serially diluted, inoculated on nutrient agar, and incubated. Identification of the isolates were done using their physical and biochemical characteristics, antibiotic susceptibility tests were done using disc diffusion method and interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** The total mesophilic bacterial counts ranged from 3.0×10^5 to 9.9×10^5 CFU/ml. These isolates were identified and tested for their sensitivity to some antibiotics which revealed that *Escherichia coli (E.coli)* were sensitive to augmentin, ampicillin, and ciprofloxacin, but intermediately resistant to chloramphenicol, *Staphylococcus aureus (S. aureus)* were sensitive to ciprofloxacin and augmentin, intermediately resistant to chloramphenicol, and resistant to ampicillin. *Bacillus* spp were sensitive to augmentin, ciprofloxacin, chloramphenicol and ampicillin, while *Pseudomonas aeruginosa* (*P.aeruginosa*) were sensitive to ciprofloxacin, augmentin, and chloramphenicol, but intermediately resistant to ampicillin. **Conclusion:** These new disposable ready-to-use cups harbored some potentially pathogenic bacteria which were resistant to some antibiotics. It is therefore recommended that pre-use hygiene practices should be done on these cups prior to use to avert the health effects of consuming these bacteria.

Introduction

Plastic is an artificially synthesized substance which has progressively become united with virtually all facets of human livelihood since it is flexible and tough; it can regularly be seen in

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human products like fabrics, prettiness products, and consumable items wrapping. The enormous commonness of plastic-based objects in the community creates a latent hazard to human well-being and to the environment. Meanwhile plastic substances substantially breakdown over some period, there is mounting apprehension over the creation of microplastics (MPs) in the process, which are plastic subdivisions that are ≤5 mm in dimension [1]. Some current research have confirmed that the incidence of MPs in the environment and in ingestible water have reaped noteworthy consideration globally due to their latent effect on human well-being [2].

The consumable substances packing factories commonly utilize papers and paperboards (PPBs) particularly for one-use items. Conferring to the Framework Regulation (EC) No. 1935/2004 of the European Union, no transference of contaminants ought to transpire from food packing substances to the consumables, nonetheless in several locations, reverse is the case. For example, results from a study carried out in India showed the existence of bacterial contamination in all the food wrapping samples tested, extending from 1.3×102 to 6.1×103 CFU/g of sample [3]. Many of those packaging samples had bacterial presence greater than the tolerable level of 2.5×102 CFU/g as established by the Food and Drug Administration [3]. The noticed bacterial contaminants were from genera *Bacillacea*, *Staphylococcus* and *Pseudomonas*, which conferring to the FDA pronouncement have remained linked with food borne illnesses [3]. Also, some microbial contaminants in food wrapping PPBs were discovered to be *Bacillus subtilis* and *Pseudomonas aeruginosa (P. aeruginosa)* which create enzymes like peroxidases and lipoxygenases that are odor-causing enzymes [3].

Nonetheless, there are non-microbiological pollutants which can be initiated from food packing like polystyrene which is utilized in foam and non-foam in the production of one-time use-throwaway vessels like cups, plates, eating/cooking utensils, etc. Such constituents are carcinogenic aromatic compounds and conferring to cautions from specialists [4].

Prolonged utilization of such items creates harmful outcomes on human healthiness [4]. In addition, there are other compounds utilized in plastic products which could usher in poisonous outcomes to the body, such encompass bisphenol, an indestructible, light, see-through substance, utilized in producing polycarbonate plastics like athletes' water flasks, food can glaze, and dental filling resources [4]. Likewise, the fat precipitations on the superficial layer of very warm fluids in plastic one-use cups are polystyrene which is a toxic substance derivative of the plastic. Similarly, dioxin is one more chemical extremely poisonous to human cells which is principally created from plastic tableware by the hotness of high-fat diets. One more example is phthalates in plastic items which leads to hormonal anomalies, birth flaws, reproductive complications, as well as elevated percentages of phthalates metabolites in the urine which aggravate asthma symptoms [5].

In Gombe metropolis, these disposable cups are commonly used to consume both hot and cold drinks without any form of pre-cleaning and without adequate knowledge regarding the health risks of using such cups, hence this study was setup to investigate the bacteriological aspect of the problem and to reduce the existing knowledge gap regarding this problem.

Methods

Sample collection

This was done using simple random sampling method [6] in which a total of 30 new unused disposable ready-to-use plastic cups were purchased from the old market (tsohuwar kasuwa) and main market (babban kasuwa) of Gombe metropolis in March 2019. Hand gloves which were disinfected with 70% ethanol on site, were worn when collecting the samples, which were immediately placed inside disinfected zippable polythene carrier bags and transported within two hours to the Microbiology laboratory of Microbiology Department Gombe State University for further processing and microbiological analysis.

Sterile cotton swab sticks which were aseptically pre-moistened with peptone water were then used to swipe the interior portion of the cups and then placed inside test tubes containing 10 mls sterile distilled water for microbial release [7].

Isolation, enumeration, and identification

The swabs were each placed in 1 ml of sterile distilled water and 10-fold serial dilutions were carried out distinctly. Subsequently, a micropipette with plastic tips disinfected in oven (to maintain integrity of the plastic) at 80^{o}C for 3 hours was used to take 100µl of sample from tube with 10^{-5} dilution and inoculated on nutrient agar plates using spread

plate technique and then incubated at 37ºC for 24 hours after which visible colonies were counted using viable count method and CFU/ml were determined later on [8].

Identification was achieved by means of physical observation of colony color and shape, then Gram's staining, and biochemical reactions that included catalase test, coagulase test, citrate test, indole test, and urease test, that were selected owing to the outcomes of the morphological characteristics and Gram's reaction of the microorganisms [9].

Gram's staining

A flame-sterilized wire loop was utilized to carry one drop of pre-suspended microbial culture to a clean grease-free glass slide where this culture was spread out using the same wire loop until an even circular thin-film was formed which had diameter of approximately 15mm. This slide was then heat-fixed, stained with crystal violet, and allowed for 60 seconds after which the stain was poured off and the slide rinsed under gentle-flowing tap water. Fixing the dye was then carried out by pouring iodine solution on the slide and allowing it for 60 seconds after which the iodine was poured off, and the slide then rinsed again using gentle-flowing tap water. Few droplets of acetone-ethanol decolorizer were then added to the slide and rinsed off after 5 seconds. This smear was subsequently counterstained using basic fuchsin solution for 50 seconds, washed off with water, and air-dried. The slide was then examined using X40 objective for assessing smear dispersal, after which the X100 oil immersion objective was used to view the microbial cells [10].

Biochemical tests

Catalase test

Three milliliters (3 mls) of hydrogen peroxide were transferred inside a sterile test tube and several colonies of the test isolate were collected using a sterile glass rod and placed inside the hydrogen peroxide, after which presentation of bubbles were sought [11].

Coagulase test

About 0.2 mls of sheep plasma were added to a loopful of the test isolate and incubated at 35^0C for 4 hours. Clotting was sought as indication for a positive coagulase test [12].

Citrate utilization test

Slopes of Simmon's citrate agar medium were prepared, dispensed in bijou bottles, and using a sterile inoculation wire, the slopes were streaked with a saline suspension of the test isolate after which the butt was stabbed, followed by incubation at 35ºC for 48 hours. Subsequently, a bright blue color was sought in the medium as an indication for positive citrate utilization [13].

Indole test

The test isolate was inoculated inside a bijou bottle containing 3 mls of sterile tryptone water, these were then incubated at 36ºC for 48 hours. 0.5 ml of Kovac's reagent was then added, followed by gentle shaking, and then looking out for a red color in the superficial level within 10 minutes [13].

Urease test

Colonies of the test isolate were heavily inoculated into 3 mls of sterile Christensen's modified urea broth inside a bijou bottle and subsequently incubated at 36ºC for 6 hours using a water bath, after which a pink color was sought in the medium as indication of positive urease test [13].

Antibiogram assay

The inocula of the identified isolates were first standardized to match the turbidity of 0.5 McFarland turbidity standard using direct colony suspension method which encompassed making a suspension of 24-hour old sub-cultured bacteria by picking with a sterile glass rod and dissolving into 2 mls of aseptic normal saline and continuously adjusting with the same saline solution until the turbidity match was reached [14]. Subsequently, the antibiogram assay was carried out using disc diffusion method which involved spread plate inoculation of 0.1ml standardized inoculum on Mueller Hinton Agar (MHA) plates then dispensing ciprofloxacin, augmentin, ampicillin, and chloramphenicol antibiotic discs aseptically on the inoculated plates except for the control which was left blank, these were subsequently incubated at 35ºC for 18 hours [15], after which zones of inhibition were recorded to the nearest whole millimeter and interpreted using guidelines for antibacterial susceptibility testing [16].

Results

The results of this study showed that the sampled disposable cups harbored some bacterial contaminants which showed varying levels of responses to antibiotic sensitivity tests.

The bacterial counts obtained from different samples collected from old market **(Table 1)** ranged from 3.3×10^5 to 9.8×10^5, while those from main market **(Table 2)** ranged from 3.0×10^5 to 9.9×10^5.

The morphological characteristics of representative isolates revealed that suspected *Escherichia coli* (*E.coli*) had large thick grayish white color on nutrient media, were rod-shaped and Gram negative. Suspected *Staphylococcus aureus (S. aureus)* had golden yellow color on nutrient agar, appeared cocci in clusters and stained Gram-positive, suspected *Bacillus* spp appeared milk colored on nutrient agar, rod shaped and stained Gram-positive, while suspected *P. aeruginosa* on nutrient agar appeared green, smooth, shiny, rod-shaped and stained Gram-negative. The biochemical reactions of the representative isolates from both old market and main market samples are shown in **table (3)**.

The sensitivity test for bacteria isolated from both old market and new market samples **(Table 4)** revealed that *E. coli* were sensitive to augmentin, ampicillin, ciprofloxacin, and intermediately resistant to chloramphenicol. *S. aureus* were sensitive to ciprofloxacin and augmentin, intermediately resistant to chloramphenicol, and resistant to ampicillin. *Bacillus* spp were sensitive to augmentin, ciprofloxacin, chloramphenicol, and ampicillin. Finally, *P. aeruginosa* were sensitive to ciprofloxacin, augmentin, chloramphenicol, and intermediately resistant to ampicillin.

Table 1. Results of total bacterial counts obtained from different packs of disposable plastic cups from old market (tsohuwar kasuwa).

Sample	Volume of inoculum used	CFU/ml
TK1	0.1ml	8.1×10^5
TK2	0.1ml	6.3×10^5
TK3	0.1ml	4.5×10^5
TK4	0.1ml	4.3×10^5
TK5	0.1ml	9.0×10^5
TK6	0.1ml	6.4×10^5
TK7	0.1ml	3.3×10^5
TK8	0.1ml	NIL
TK9	0.1ml	3.9×10^5
TK10	0.1ml	9.8×10^5
TK11	0.Iml	NIL
TK12	0.1ml	5.5×10^5
TK13	0.1ml	6.1×10^5
TK14	0.1ml	7.7×10^5
TK15	0.1ml	7.9×10^5

Table 2. Results of total bacterial counts obtained from different packs of disposable plastic cups from main market (babban kasuwa).

Sample area	Volume used	Bacterial count (CFU/ml)
BK1	0.1ml	NILL
BK2	0.1ml	NILL
BK3	0.1ml	NILL
BK4	0.1ml	5.5×10^5
BK5	0.1ml	7.8×10^5
BK6	0.1ml	9.0×10^5
BK7	0.1ml	4.0×10^5
BK8	0.1ml	3.0×10^5
BK9	0.1ml	7.5×10^5
BK10	0.1ml	4.5×10^5
BK11	0.1ml	NILL
SK12	0.1ml	9.9×10^5
BK13	0.1ml	7.8×10^5
BK14	0.1ml	3.1×10^5
BK15	0.1ml	4.4×10^5

Table 3. Biochemical reactions of presumed isolates

Presumed organisms	CA	IN	CT	CO	OX	UR	Organisms identified
E. coli	-	+	+	-	-	-	*E. coli*
S. aureus	+	-	-	+	-	-	*S. aureus*
P aeruginosa	+	-	+	-	+	+	*P. aeruginosa*
Bacillus spp	+	-	+	-	-	_-	*Bacillus spp*

KEY: CA=Catalase test, IN=Indole test, CT=Citrate test, OX=Oxidase test, UR= Urease test, CO=Coagulase test, - = Negative, + = Positive

Table 4. Mean values and interpretation of inhibition zones from the sensitivity test.

Organisms	CH	CPX	AU	AM
E. coli	15mm (I)	26mm (S)	20mm (S)	17mm (S)
S. aureus	16mm (I)	29mm (S)	28mm (S)	20mm (R)
Bacillus spp	23mm (S)	21mm (S)	26mm (S)	26mm (S)
P. aeruginosa	25mm (S)	24mm (S)	26mm (S)	17mm (I)

KEY: CH=Chloramphenicol, CPX=Ciprofloxacin, AU=Augmentin, AM=Ampicillin, S=sensitive, I=intermediate, R=resistant

Discussion

The bacteria isolated from these plastic cups are believed to be contaminants that originate from either the industrial packaging stage or through unhygienic commercial handling, **Rana et al.** [17] previously isolated some bacteria from samples of food packaging items in Jashore, Bangladesh. The identification and typing of these isolates are in-line with the works of **Christopher** [18] who described how to identify bacteria using their morphological characteristics, and with **Tassadaq et al.** [19] who identified some bacteria using their biochemical properties. The interpretations of the sensitivity tests were based on the guidelines for antimicrobial susceptibility testing produced by Clinical and Laboratory Standards Institute [16].These sensitivity patterns of resistance observed are believed to be owing to natural outcomes of evolution which makes these bacteria to make adaptations necessary for tolerance/survival in response to various unfavorable conditions especially exposure to non-bactericidal doses of antibiotics which are seriously been misused [20], these findings are in agreement with reports of **Laganà** [21] who had previously isolated antibiotic resistant bacteria from consumable items and their related surroundings.

The disposable ready-to-use plastic cups used in Gombe metropolis are contaminated with high levels of bacteria which can lead to transfer of contamination to food items and subsequent consumption by individuals, thus leading to illnesses which have detrimental effect on the quality of life and productiveness of members of society. It is thus recommended that the good manufacturing practices (GMP) of these disposable ready-to-use plastic cups industries be evaluated and reviews be made to any industrial practices found to be below standard.

The limitations of the study included lack of information such as manufacturer name, address, and batch number on the sealed packs of these disposable cups, which hampered any effort at evaluation of the manufacturers' GMP.

Conflicts of interests

The authors report no conflicts of interest. All authors have approved the final article.

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Author contributions

1. Umar Abdullahi Tawfiq: Antibiogram assay
2. Bashir Mohammed: Biochemical tests
3. Anita Aliyu: Sample collection, Isolation
4. Abdulrasheed Mansur: Morphological identification
5. Puma Hamidu Umar: Biochemical tests
6. Shu'aibu Isa: Literature review, Article writing
7. Kawuwa Abubakar Usman: Pure culture, Standardization of inocula

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