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Original Research Article

Bacteriostatic efficacy of commonly used detergents against carbapenemase-producing *Escherichia coli* from food contact surfaces in Nasarawa State, Nigeria

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ABSTRACT

Background: Carbapenemase-producing *Escherichia coli* (CPEC) has been an issue of public health concern due to high resistance to major antibiotics. This pathogen can be acquired through the consumption of contaminated foods. The use of detergents for the control of pathogens in food contact surfaces is commonly practiced by most food vendors. This study is aimed at assessing the efficacy of some commonly used detergents against CPEC from food contact surfaces in Nasarawa State, Nigeria.
Materials and Methods: A total of 924 swab samples from food contact surfaces (246 each from plates, cups and spoons and 186 from tabletops) in various food vending outlets were screened for phenotypic identification of CPEC using Imipenem (30μg) and Meropenem (10μg) antibiotics susceptibility disk diffusion method. Tubes of varying concentrations of test detergents (1:140, 1:160, 1:180, 1:200 and 1:220) were seeded against phenol as standard with varying concentrations of 1:50, 1:60, 1:70 1:80 and 1:90.
Results: Hawkers outlets had highest CPEC isolation frequencies among swab samples and *E. coli* isolates with values of 3.33% and 7.70% respectively while cups and spoons surfaces had highest samples (1.63%) and isolates (7.69%) frequencies respectively. Overall CPEC isolation frequencies were 1.30% and 6.00% among the screened samples and *E. coli* isolates respectively. Though there was significant difference (p<0.05) in the frequency values among the vending outlets, the contact surfaces had no significant difference at p>0.05. Highest phenol coefficient value of 2.6 was obtained for MFD while SLD and LPD had 2.3 and 1.7 respectively. The difference in the phenol coefficient values among the detergents were statistically insignificant (p>0.05).
Conclusion: All detergents used in this study were effective against CPEC isolated from the food contact surfaces. Using locally produced detergents in appropriate concentrations especially for low-income earners is quite promising and is a good alternative to other branded detergents.

1. Introduction

All food vending outlets have food contact surfaces such as utensils, worker's hands, worker's clothing, all equipment, facilities and packaging materials. Pathogens

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can therefore be transmitted through these surfaces.[1] Many cases of foodborne illnesses have not only been linked to contaminated raw ingredients usage, improper cooking and control of temperature but also cross contamination of food through food contact surfaces.[2]

In food service establishments, it has been reported that the main sources of microbial contamination are food contact surfaces.[3,4] The moist nature of food contact surfaces, which become wet during preparation and serving of food, provides suitable conditions for microbial growth.[5,6] Soil and water has been reported to harbor *E. coli* which can be transferred to food contact surfaces during food preparation.[7] Good hygienic practices therefore limit the suitable conditions for the growth of these organisms.[5,8,9] Patronizing food from unhygienic food outlets increases the potential for foodborne infections such as diarrhea or other colibacillosis cases.[10,11] The preparation and exposure during sales, storage processes and mode of handling play a significant role in the proliferation of the pathogens to a certain level in food contamination hence the cleanliness of food contact surfaces could be an indicator of a good hygiene level.[12] Contaminated food can endanger public health by causing various acute and chronic foodborne illness through pathogenic microbes or toxic substances present in them.[13]

Foodborne disease caused by carbapenemase-producing *E. coli* is of public health concern, since these species have evolved defense mechanisms against antimicrobial agents, which have traditionally been used as a last resort in modern clinical science.[14,15] Large groups of carbapenemases have been identified, such as ambler class A, B, and D thus making the issue of treatment a problem.[16] It has been discovered that carbapenemase-producing *E. coli* are not only present in clinical settings such as hospitals and clinics, but the organism has also been isolated from food vendors, meat vendors, and domestic animals such as chickens and pigs.[17] Carbapenemase is the most flexible in the β-Lactamase family.[18] Carbapenemase-producing *E. coli* can effectively colonize food vendors' hands, counters from which food is served, table tops on which customers eat and other equipment such as knives, spoons and other cooking utensils.[19]

Many detergents are specifically made to reduce microorganisms from surfaces and aid good hygiene. Most detergent products work effectively to reduce microorganisms by introducing them on the materials for about 5 minutes.[20,21] Modes of application and the presences of food particles reduces the efficacies of some detergents against pathogenic microorganisms.[22]

Although carbapenemases have been known to be new and a potentially emerging problem in food production and sales, the prevalence of carbapenemase-producing bacteria from food vending outlets has been scarcely reported.[23] So far, most of the epidemiological studies and the significance of Carbapenemase-producing *E. coli* have been focusing on human and food animals with little information on environmental involvement such as the case with food contact surfaces. Hence, this study was aimed at accessing the bacteriostatic potentials of commonly used detergents against carbapenemase-producing *Escherichia coli* from food contact surfaces in Nasarawa State, Nigeria

2. Materials and Methods

2.1. Sample collection

A total of 924 swab samples were collected from food contact surfaces (246 each from spoons, plates and cups and 186 from table tops,) of food vending outlets aseptically using sterile swabs sticks. The samples were appropriately labeled and immediately transported to the Microbiology Laboratory, Federal University of Lafia, for further analyses.

2.2. Isolation and identification of Escherichia coli

The process for isolating *E. coli* was carried out as described by Cheesbrough.[24] Each sample was spread onto MacConkey agar with sterile swap sticks and incubated for 24 hours at 37 °C. Suspected *E. coli* colonies with pink coloration were sub-cultured on Eosin Methylene Blue agar with a sterile inoculating loop and incubated for 24 hours at 37 °C under aerobic conditions. *E. coli* colonies with a greenish metallic sheen were selected, placed on nutrient agar slant, and refrigerated at 4^{o}C for biochemical analysis.

2.3. Identification of Escherichia coli isolates

Identification and characterization of bacteria isolates was carried out using as described by Cheesbrough.[24] The isolates were identified using cultural, morphological and Gram staining characteristics. Biochemical tests characteristic to *Escherichia coli* were carried out namely Urease, Motility, Indole, Methyl Red Voges-Proskauer (MRVP), Citrate, and sugar fermentation on Triple Sugar Iron agar.

2.4. Phenotypic identification of carbapenemase-producing Escherichia coli

2.4.1. Inoculum preparation

A stock culture of each confirmed *Escherichia coli* isolate was resuscitated by inoculating a 24- hours old culture of the test organism into sterile peptone water and incubated for 2 hours to allow the organism reaches itss log phase of growth. This was then diluted to match the McFarland turbidity standard of 0.5 which contains approximately 1.5 x 10^{8} cfu/ml (Cheesbrough, 2008).

2.4.2. Screening for carbapenemase-producing Escherichia coli

The preparesd inoculum of each *Escherichia coli* isolate was subsequently screened for carbapenem resistance by Kirby Bauer disk diffusion method following the Clinical and Laboratory Standard Institute guidelines using imipenem (30 μg) and meropenem (10μg) antibiotics disks (Oxoid UK) as standards.[25] Isolates which showed inhibition zone diameter of ≤21mm for imipenem and ≤ 19 mm for meropenem were considered as potential carbapenemase producers

2.5. Phenol coefficient test

This was performed according to the method of Sondawale et al.[26] with slight modification. Commonly used cleaning agents (Morning Fresh, Sunlight and Locally Produced Liquid Detergents) were prepared at various concentrations using ditch- plate technique. Tubes of varying concentrations of test detergents (1:140, 1:160, 1:180, 1:200 and 1:220) were prepared alongside phenol with varying concentrations of 1:50, 1:60, 1:70, 1:80 and 1:90 as standard. A loopful of *E. coli* was inoculated in each dilution. Dilutions were vortexed to ensure proper contact with the disinfectant at intervals of 2, 5, 7 and 10 minutes. Subsequently, a loopful of each suspension was transferred aseptically into the nutrient agar plate and incubated at 37 ^{0}C for 48 hours to observe for growth. The test detergent agents with a phenol coefficient >1 have a greater efficacy to phenol while those with phenol coefficient equal to or < 1 have lesser antimicrobial activity compared to phenol.

PC= Highest dilution of disinfectant that kills the test organism (MICD) in 10 mins, not in 5min

Highest dilution of phenol that kills the test organism (MICP)S in 10 mins, not in 5 mins

Where PC = Phenol Coefficient.

2.6. Data analysis

SPSS version 20 (Statistical Package for Social Sciences) was used for statistical analysis of the data. One way and a two-way ANOVA (analysis of variance) were used to determine differences in the group means. Statistical importance was set at 0.05 confidence level.

3. Results

3.1. Occurrence of carbapenemase-producing E. coli from various food contact surfaces

The frequency of occurrence of carbapenemase-producing *E. coli* (CpEC) from various food contact surfaces in the various food vending outlets sampled is shown in Table 1. CpEC had highest frequency of 1.63% among the cup samples while the overall sample frequency was 1.30%. However, among the positive *E. coli* isolates, CpEC had highest frequency of 7.69% from the spoon isolates with an overall isolates frequency of 6.00%. No statistically significant difference (p>0.05) was observed among the samples and isolates frequencies.

3.2. Occurrence of carbapenemase-producing E. coli isolates from various food contact surfaces in relation to food vending outlets.

Table 2 showed result of the incidence of carbapenemase-producing *E. coli* isolates from foodcontact surfaces analyzed in relation to food vending outlets. Highest samples and isolates frequencies of 3.33% and 7.70% respectively were obtained from hawkers outlet while no *E. coli* was isolated from Eateries and Hotels outlets. There were significant differences (p<0.05) among the samples and isolates frequencies from the various food vending outlets.

3.3. Occurrence of Carbapemenase – producing E. coli from food contact surfaces in relation to both locations

The incidence of carbapenemase-producing *E. coli* from various food contact surfaces in relation to both locations is shown in Figure 1. Samples and isolates frequencies of 1.34% and 6.41% respectively were obtained from food contact surfaces in Lafia Metropolis while 1.74% and 5.74% respectively were obtained from food contact surfaces in Nasarawa Eggon. Though a significant difference (p<0.05) was observed in the prevalence of *E. coli* from food contact surfaces between both locations, there were no significant differences (p>0.05) among the samples and isolates frequencies of carbapenemase-producing *E. coli* from the surfaces of both locations.

3.4. Phenol coefficient of dish washing soap against carbapenemase-producing E. coli isolated from various food contact surfaces

Table 3 showed the results of Phenol Coefficient Test carried out using washing detergents frequently used on food contact surfaces by food vendors. Morning Fresh Detergent (MFD) had the highest phenol coefficient value of 2.6 against carbapenemase-producing *E. coli* isolates from all food contact surfaces followed by Sunlight Detergent (SLD) and Locally Produced Detergent (LPD) with 2.3 and 1.7 respectively. There was however no significant association (p>0.05) between the phenol coefficient values and the type of food contact surface of the isolates.

3.5. Phenol coefficient of dish washing soap against carbapenemase-producing E. coli isolated from various vending outlets

Table 4 showed the results of the Phenol Coefficient test carried out using frequently used food contact surfaces detergents against carbapenemase-producing *E. coli* isolates based on different food vending outlets. Highest phenol coefficient value of 2.6 was obtained using MFD and carbapenemase-producing *E. coli* isolates from Restaurants, Street vendors and Hawkers outlets while similar results of 2.3 and 1.7 were obtained using SLD and LMD respectively. However, no significant association was obtained (p>0.5) between the phenol coefficient values and the vending outlet of the isolates.

4. Discussion

Carbapenemase-producing *Escherichia coli* has become a global public health issue since the carbapenem antibiotics are among the most critically important antimicrobials for the treatment of infections in humans.[23] Good hygiene practices also known as Good Manufacturing Practices (GMP) is vital to guarantee food safety, as compromising this good practice almost always results in the establishment and proliferation of pathogenic organisms.[8]

This study has revealed a high contamination level of carbapenemase-producing *E. coli* (CPEC) contamination frequency value of 1.63% in cups swab samples and an overall contact surfaces frequency of 1.30%. CPEC frequency among the *E. coli* isolates had the highest value of 7.69% among the spoon swab isolates and an overall contact surfaces frequency value of 6.00%. However, no statistically significant difference was observed in the CPEC frequency among both groups. This may be due to the fact that same individuals were involved in handling of the various food contact surfaces which accounted for similar CPEC isolation frequencies. However, Ssekatawa et al.[27] reported a carbapenem-resistant *E. coli* (CREC) prevalence of 22.80% from surfaces in Uganda tertiary hospitals and 22.8% of CRE found among Carbapenem-resistant enterobacteriaceae from chicken.[28] These values were found to be higher than those obtained from this study. The significant differences observed were due to the differences in the sampled environments.

The prevalence of carbapenemase-producing *E. coli* (CPEC) from different food contact surfaces in relation to food vending outlets is shown in Table 2. Results of this study indicated the highest swab samples and isolates frequencies of 3.33% and 7.70% respectively obtained from Hawkers food contact surfaces. No CPEC was isolated from restaurants and hotels and significant differences in the frequency values from the various food outlets were observed at p<0.05. The differences in the prevalence values may be due to the high differences in food vending environments and hygienic status of the food vendors from the various outlets. Similar studies by Onyidibe et al.[29] reported 2.4% of clinical isolates of ESBL-producing *E. coli* exhibiting carbapenem resistance. However, a related study by Beshiru et al.[30] reported a high percentage of 75.8% from processed read-to-eat foods outlets in Yenegoa, Nigeria having MARI values >2. These results are indications that food vending outlets can serve as routes for the transmission of CPEC.

Results from both locations showed higher swab samples isolation frequency value of CPEC obtained from Nasarawa Eggon (1.74) while higher frequency values of CPEC among *E. coli* isolates was obtained from food contact surfaces in Lafia Metropolis (6.41%). However, no significant difference (p>0.05) was observed among both groups of frequency values obtained from the two locations (Figure 1). Similar behavioural activities with respect to drug administration and usage by residents of both communities may account for the similar isolation frequency of CPEC from food contact surfaces in the communities.

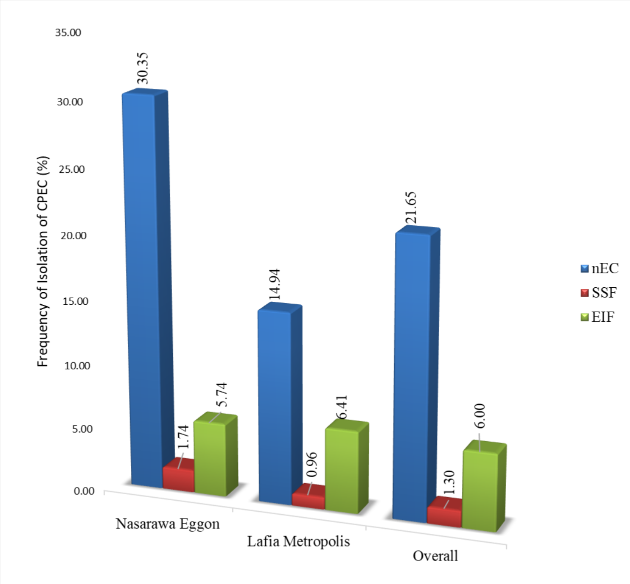

Fig. 1: Prevalence of CPEC from food contact surfaces in both locations
nEC = No. of positive *E. coli* samples; SSF = Swap Samples Frequency; EIF = Frequency among *E coli* Isolates; CPEC = Carbapenemase-producing *E. coli*

An ideal dish washing detergent should have a broad antimicrobial spectrum, should be non-irritating, less toxic, noncorrosive and most especially inexpensive. This study has revealed the phenol coefficient of some dish washing detergents frequently used by vending outlets in washing most of their food contact surfaces as very effective against *E. coli*. All detergents had phenol coefficients greater than 1.0 with values of 2.6, 2.3 and1.7 for MFD, SLD and LMD respectively. This correlates with the findings of Raut et al.[31] who reported the efficacy of different soaps which all had high phenol coefficient values against *E. coli*. However, the high prevalence of *E. coli* isolated from food vending outlets that used these detergents is still a major course for concern as this might be due to the fact that the food

Table 1: Occurrence of Carbapenemase-producing *E. coli* isolates from various contact surfaces in food vending outlets

Contact Surfaces	N	n EC (%)	Sample Frequency $\frac{nCpEC}{N}(\%)$	Isolates Frequency $\frac{nCpEC}{n\_EC}(\%)$
Plates	246	51(20.73)	$\frac{3}{245}(1.22)$	$\frac{3}{51}$ (5.88)
Cups	246	57 (23.17)	$\frac{4}{246}$ (1.63)	$\frac{4}{57}(7.02)$
Spoons	246	26(10.57)	$\frac{2}{246}(0.81)$	$\frac{2}{26}(7.69)$
Table tops	186	66(35.48)	$\frac{3}{186}(1.61)$	$\frac{3}{66}(4.55)$
Total	924	200 (21.65)	$\frac{12}{924}(1.30)$	$\frac{12}{200}(6.00)$
		P<0.05	P>0.05	P>0.05

N = Total no. of screened samples; nEC= No. of positive E.coli samples; nCpEC = No. of positive Carbapenemase-producing *E.coli* isolates

Table 2: Incidence of Carbapemenase – producing *E. coli* from difference vending outlets

Vending Outlets	N	nEC (%)	Samples Frequency $\frac{nCpEC}{N}(\%)$	Isolates Frequency $\frac{nCpEC}{n\_EC}(\%)$
Restaurants	240	47 (19.58)	$\frac{2}{240}(0.83)$	$\frac{2}{47}$ (4.26)
Street vendors.	240	75 (31.25)	$\frac{4}{240}$ (1.67)	$\frac{4}{75}(15.33)$
Hawkers	180	78 (32.50)	$\frac{6}{180}$ (3.33)	$\frac{6}{78}(7.70)$
Eateries	192	0 (0.00)	0 (0.00)	0(0.00)
Hotels	72	0 (0.00)	0 (0.00)	0 (0.00)
Total	924	200 (21.65)	$\frac{25}{924}(1.30)$	$\frac{12}{200}$ (6.00)
		P<0.05	P<0.05	P<0.05

N = Total no**.** of screened samples; nEC=No. of positive *E. coli* samples; nCpEC = No. of positive Carbapenemase-producing *E. coli* isolates *E. coli* samples; SSF = Swap Samples Frequency; EIF = Frequency among *E coli* Isolates; CPEC = Carbapene *E. coli.*

Table 3: Phenol coefficient of dish washing detergents against carbapenemase-producing *E. coli* isolated from various contact surfaces

Detergents	**Spoons**	**Cups**	**Plates**	**Table tops**	**F**
MFD					
MICD	1:180	1:180	1:180	1:180	1.8020
MICP	1:70	1:70	1:70	1:70	
PC	2.6	2.6	2.6	2.6	
SLD					
MICD	1:160	1:160	1:160	1:160	
MICP	1:70	1:70	1:70	1:70	
PC	2.3	2.3	2.3	2.3	
LPD					
MICD	1:120	1:120	1:120	1:120	
MICP	1:70	1:70	1:70	1:70	
PC	1.7	1.7	1.7	1.7	

MICD= Minimum Inhibitory Concentration of Detergent; MICP = Minimum Inhibitory Concentration of Phenol; PC= Phenol Coefficient; **MFD=** Morning Fresh Detergent; **SLD=** Sunlight Detergent; **LPD=** Locally Produced Detergent

handlers most often dilute these detergents higher than the dilutions used in this study.

The regular implementation of good hygienic practices are primary preventive measures hence the monitoring of their effectiveness will not only provide an early warning of potential problems but also evidence of due diligence.

5. Conclusion

Some food contact surfaces in food vending outlets analyzed in this study were shown to harbor carbapenemase-producing *Escherichia coli* though none was isolated from food contact surfaces in eateries and hotels. All detergents in the panel were effective against carbapenemase-producing *Escherichia coli* isolated from the contact surfaces with their phenol coefficients >1.0. This study recommends the use of these detergents in the appropriate concentrations for the effective cleaning of food contact surfaces in various food vending outlets.

6. Authors' Contributions

This research was carried out with the total collaboration of all the authors. Author NTD and JFN conceived, designed and wrote the protocols and first draft of this study. Authors DAH, SHN, KUH and SRA compiled the necessary literature and statistical analyses while authors VKF, MIA, IOI and YA contributed to the laboratory analyses. All

Table 4: Phenol coefficient of food surfaces detergents against carbapenemase- producing *E. coli* isolated from food contact surfaces based on various vending outlets

Detergents	Restaurant	Hawkers	Street vendors	F
MFD				
MICD	1:180	1:180	1:180	1.8020
MICP	1:70	1:70	1:70	
PC	2.6	2.6	2.6	
SLD				
MICD	1:160	1:160	1:160	
MICP	1:70	1:70	1:70	
PC	2.3	2.3	2.3	
LPD				
MICD	1:120	1:120	1:120	
MICP	1:70	1:70	1:70	
PC	1.7	1.7	1.7	

MICD= Minimum Inhibitory Concentration of Detergent; MICP = Minimum Inhibitory Concentration of Phenol; PC= Phenol Coefficient; **MFD=** Morning Fresh Detergent; **SLD=** Sunlight Detergent**; LPD=** Locally Produced Detergent

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8. Conflict of Interest

All items used in this research were obtained locally and mainly used in our area of research. There is therefore no conflict of interest between the authors and producers of such items.

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