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ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND PREVALENCE OF ESBLs AMONG *E.coli* ISOLATES RECOVERED FROM PEOPLE WORKING IN HOSPITALITY INDUSTRY WITHIN NAIROBI, KENYA

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ABSTRACT

Objective: To determine the antimicrobial susceptibility profiles with key focus on ESBL-producing *E.coli* strains isolated from participants working in the hospitality industry in Nairobi, Kenya

Study design: A cross sectional descriptive study.

Setting: Centre for Microbiology Research at Kenya Medical Research Institute, Nairobi.

Subjects: A total of 323 food-handlers working within Nairobi County were recruited.

Results: High resistances were recorded for Sulfamethoxazole/Trimethoprim (70%), Ampicillin (44.6%), Streptomycin (42%) and Tetracyclines (41%) while Imipenem and Cefepime were effective against 99% of the isolates. A third of all isolates were multidrug resistant. The prevalence of ESBLs was 3.4% while CMT, IRT and pAmpC-phenotypes accounted for <2%. About 1% of the ESBL-producers were also resistant to ciprofloxacin and gentamicin. The *bla*_{TEM} accounted for 37%, *bla*_{SHV} (25%), *bla*_{CTX-M} (12%) and *bla*_{OXA-1} gene (7%). Majority of MDR strains were obtained from young individuals working in middle class hotels. Genetic relatedness of the MDR isolates was apparently influenced by the resistance profiles, hotel type and clinical characteristics.

Conclusion: This study revealed that apparently healthy people working in the hospitality industry carry MDR *E. coli* that could potentially be transmitted to the general public. Infections by such strains could result in limited treatment options increasing the chances of patient mortality. Therefore, there is need to contain the spread of such strains through promotion of rational use of antimicrobials, proper hygiene and certification of food handlers based on proper laboratory investigations.

INTRODUCTION

Increased urbanization, industrialization as well as population increase in cities has led to an increase in consumption of food in public eating-places such as hotels and restaurants. It has been shown that more than 40% of Nairobi residents consume foods made outside their homes and this raises possibilities of food poisoning especially if the food is unhygienically prepared or handled by people who are infected [1]. The chances of food contamination depend heavily on the health status of food handlers, their hygiene, as well as their knowledge, attitude and practices [2]. Thus, an infected food handler and in particular those working in the hospitality industry may pose a real threat to health especially to those patronizing their eateries [3][4].

Pathogenic bacteria, especially the multi-drug resistant (MDRs) strains that are resistant to β -lactam antibiotics are of special interest because this class of antimicrobials is heavily relied upon in the treatment of various infections especially the invasive ones. Loss of potency to this class of antimicrobials may lead to reduction in treatment options, raise associated healthcare costs, and increase the severity and duration of infections [5][6].

An increase in the proportion of enteric strains that are resistant to β -lactams especially to 3rd and 4th generation cephalosporins (a class of β -lactams) due to ESBL production is a major concern in Kenya. This rise in resistance is partially associated with ineffective enforcement of laws guiding their dispensation, access and usage [7]. ESBL strains encountered in Kenya, especially those encountered in hospital settings are also more likely to exhibit resistance to fluoroquinolones and newer aminoglycosides (Amikacin and

gentamicin), two classes of antimicrobials frequently used for treatment of infections caused by ESBL-producers [8].

Past studies conducted in Kenya have not determined the prevalence of MDR strains among food-handlers working in different categories of hotels in Nairobi and the proportion of those exhibiting special resistance phenotypes such as the ESBL-producers is yet to be determined. Since *E. coli* is a good indicator species for transmission and antimicrobial resistance in community settings [9], fecal samples and socio-demographic characteristics were collected from 323 food-handlers working in different categories of hotels in Nairobi. Susceptibility profiling and phylogenetic relatedness of isolates obtained from participants were analyzed using culture, molecular and in silico analysis and presented descriptively.

This study sought to determine the antimicrobial susceptibility profiles with key focus on ESBL-producing *E.coli* strains isolated from people working in various food establishments in Nairobi, Kenya (from low class to high class hotels).

MATERIALS AND METHODS

In this cross sectional study, fecal samples were collected from 323 apparently healthy food-handlers working in different hotels located in various parts of Nairobi County, the Kenyan capital (figure 1) and who personally sought medical certification at the Centre for Microbiology Research (CMR). Simple random sampling technique was used to collect the samples whereby every third individual was selected to participate in the study.

The hotels in which the participants worked in were conveniently classified into

three classes based on their food prices, the diversity of their facilities and services rendered. Low class hotels (LCH) were conveniently categorized as food stalls along the roadside majorly made of makeshift structures and selling food with no extra social or recreational facilities. The average cost of tea and a baked snack costs less than a dollar in the LCH. Middle class hotels (MCH) included restaurants and cafeterias operating in permanent structures including those of up the three star category and these generally selling fast-foods with prices ranging from more than a dollar for the same kind of snacks and tea. The high class hotels (HCH) comprised of hotels rated from four stars and above. The areas where the participants resided were conveniently categorized into the four cardinal directions (East, West, North or South) based on the geography of Nairobi.

A pre-tested structured questionnaire was used for collection of data regarding socio-demographic characteristics, hygiene practices and clinical history of consented participants.

The study was approved by the Kenya Medical Research Institute (KEMRI) Scientific Ethical Review Unit (IRB approval number SCC No: 3084) in accordance with the code of ethics for biomedical research involving human samples.

Stool specimen was collected from each food-handler in sterile universal containers. All the samples were first cultured into buffered peptone water and then sub-cultured on MacConkey Agar and incubated. *E.coli* strains were identified using standard procedures [10]. Antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion technique against; Ampicillin (AMP, 10 μ g), Imipenem (IPM, 10 μ g), Cefotaxime (CTX, 30 μ g), Ceftazidime (CAZ, 30 μ g), Aztreonam (ATM, 30 μ g), Cefoxitin (FOX,

30 μ g), Cefepime (FEP, 30 μ g), Amoxicillin-Clavulanic (AMC, 30 μ g), Ciprofloxacin (CIP, 10 μ g), Nalidixic acid (NA, 10 μ g), Tetracycline (TE, 30 μ g), Gentamicin (CN, 10g), Sulfamethoxazole/trimethoprim (SXT, 23.75 μ g: 1.25 μ g), Streptomycin (S, 25 μ g) and Chloramphenicol (C, 30 μ g).

Inhibition zones were interpreted using the Clinical and Laboratory Standards Institute guidelines [11]. For ease of analysis, strains with intermediate zone diameters were grouped together with the resistant ones as detailed in a past study [8]. Only isolates showing synergy zones between amoxicillin/clavulanic and a third generation cephalosporin were picked as potential ESBL-producers [12] whereas others were further classified into fully susceptible, resistance to less than three classes of antimicrobials and MDR strains.

Strains exhibiting resistance to any β -Lactam were further screened for selected *bla* genes encoding for TEM, CTX-M, SHV and OXA enzymes that have been identified as the most common β -Lactamases in Enterobacteriaceae using conventional PCR method. To determine clonal relatedness of the strains, strains were selected to represent participant of different socio demographic and clinical characteristics. Antimicrobial resistance phenotypes were typed using the (GTG)₅-PCR scheme and the banding patterns analyzed using the gel compar II software (Bionumerics, Belgium).

STATISTICAL ANALYSIS

A chi-square statistical test was used to analyze bivariate data and all results were interpreted at 95% confidence interval and presented in tabular and graphical formats.

RESULTS

Out of the total 323 food handlers surveyed, the ratio of females to males was near parity (49% and 51% respectively). The participants worked in hotels located across the city as shown in Figure 1.

Majority (75%) of the participants were between the ages of 18-30 years. A high proportion 245 (76%) of the participants worked in the Middle Class Hotels (MCH), 51(16%) in High-Class Hotels (HCH) while 27(8%) worked in Low-Class Hotels (LCH). Majority of the participants 177(55%) had a tertiary education and only a small proportion (<1%) were either illiterate or only had only a primary school education (3%).

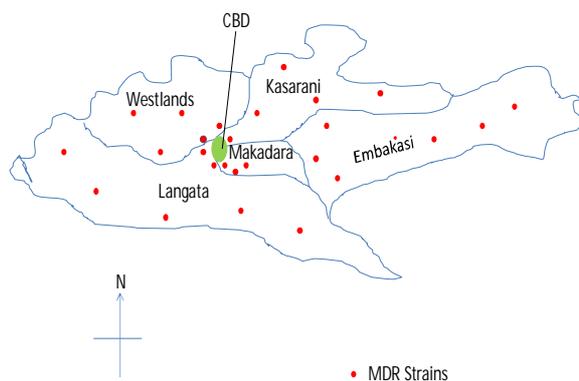
Over 70% of those sampled had no valid medical license to operate as food-handlers and another 33% had not received any formal training regarding food hygiene and safety. About (67%) of the food-handlers revealed that they normally buy medications or use

alternative medicines (self-medicate) when sick without consulting a clinician and with a significant proportion (11%) indicated that they did not complete the dosage as required (Supplementary Table 1-1).

A significant proportion of the isolates were resistant to sulfamethoxazole/trimethoprim (SXT, 70%), ampicillin (AMP, 44.6%), streptomycin (S, 42%) and tetracyclines (TE, 41%). Resistance to third generation cephalosporins [Ceftazidime (CAZ) and Cefotaxime (CTX)] was about 6%. Low level of resistance was observed for cefepime (<1%), imipenem (<1%), gentamicin (1.9%) and ceftoxitin (2.8%). The prevalence of isolates exhibiting the classical ESBL phenotype was at 3.4% with almost equal proportions among either genders (men 3.6%; and female 3.2%). Moreover, only 3% of all isolates analyzed were ESBL-producers and also exhibited ciprofloxacin resistance, Figure 2.

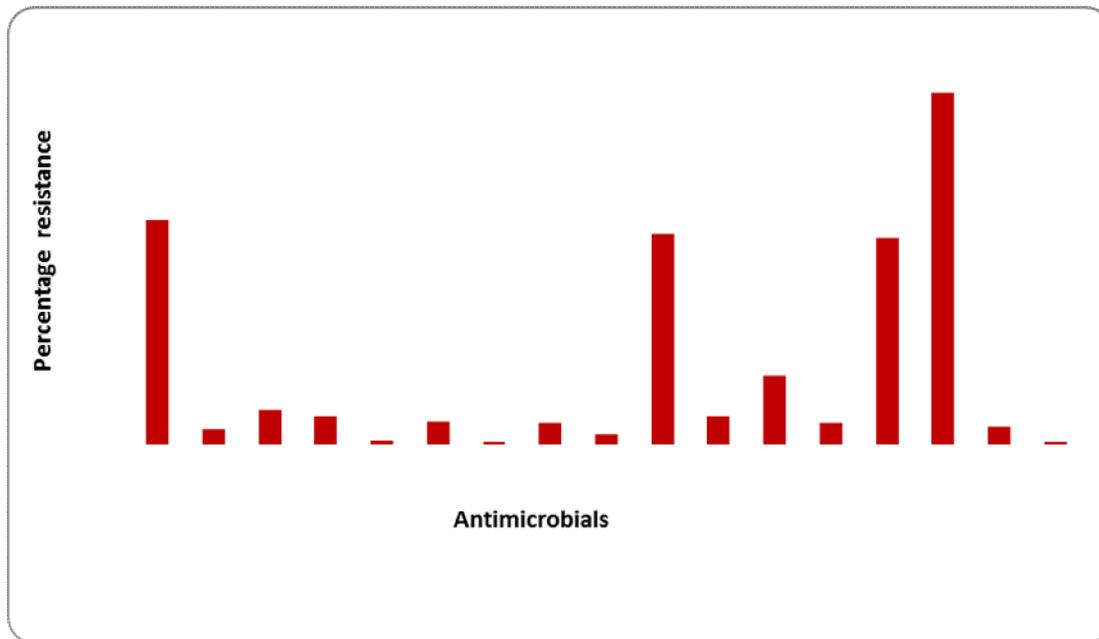
Figure 1

A map of Nairobi County showing the distribution of MDR strains recovered from apparently healthy food-handlers working in various food establishments within the County



CBD: Central Business District

Figure 2
General Resistance pattern of *E. coli* isolates recovered from healthy food-handlers working in various food establishments within Nairobi County on selected antimicrobials



AMP-Ampicillin, **IPM**-Imipenem, **CTX**- Cefotaxime, **CAZ**-Ceftazidime, **ATM**-Aztreonam, **FOX**-Cefoxitin, **FEP**-Cefepime, **AMC**- Amoxicillin-Clavulanicacid, **CIP**-Ciprofloxacin, **NA**-Nalidixicacid, **TE**-Tetracycline, **CN**-Gentamicin, **S**-Streptomycin, **SXT**-Sulfamethoxazole/trimethoprim, and **C**-Chloramphenicol, **ESBL**-Extended Spectrum β -lactamases

ESBL+CIP+CN: Isolates that exhibited ESBL phenotype and were also resistant to both ciprofloxacin and gentamicin antimicrobials

A third of all isolates 97(30%) were MDR, majority of which were isolated from individuals working in middle class hotels (MCH) around the Central Business District (CBD). The carriage of this phenotype among isolates obtained from females was slightly higher (33%) than of those obtained from males (27%). This MDR phenotype among strains obtained from participants aged between 18 and 30 years was not statistically different from those obtained from participants above the age of 30years ($p:0.850$, $OR:0.949$, $CI:0.31-1.7$) indicating that the dissemination of MDR strains is not influenced by age.

The type of medication (hospital treatment vs. self-medication) observed among the study population did not influence the carriage of isolates exhibiting the MDR phenotype ($p: 0.482$, $OR: 0.834$, $CI: 0.489-1.427$). The ESBL phenotype was recorded in 5% and 2% among individuals with secondary and tertiary education respectively, whereas none of such strains was recovered from illiterate participants.

A high proportion (30%) of isolates from individuals lacking a food-handlers' certificate were MDR with about 3% of such isolates exhibiting the ESBL phenotype. Another 1% of isolates from this category of

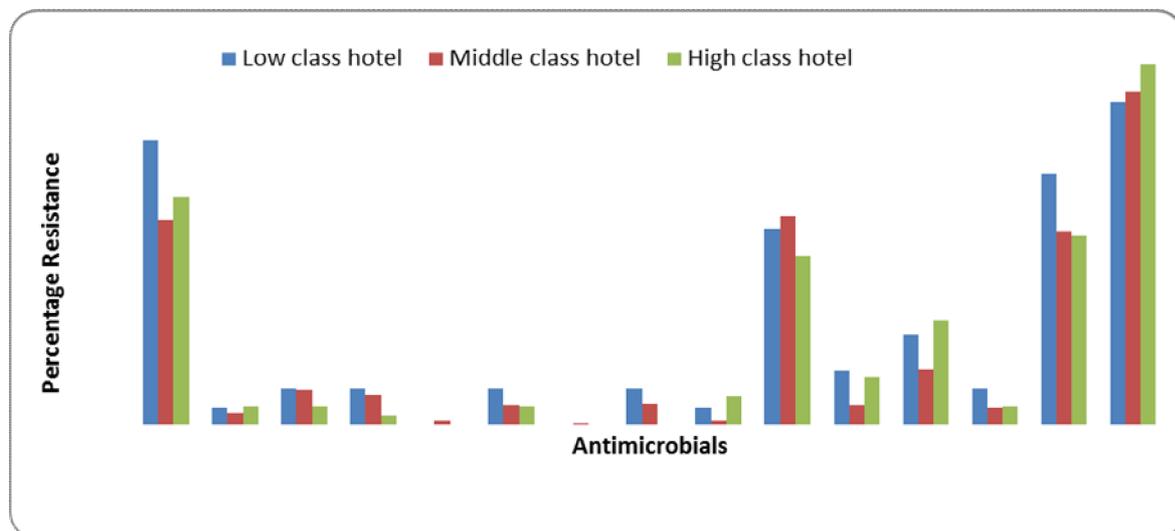
participants were not only ESBL-producers but also exhibited combined resistance to ciprofloxacin and gentamicin [ESBL+Ciprofloxacin+Gentamicin phenotype].

High resistance of $\geq 40\%$ was recorded for ampicillin, streptomycin, tetracycline and sulfamethoxazole/trimethoprim regardless of the type of hotel the food handlers worked in.

In contrast, low resistances of below 5% were recorded against imipenem, aztreonam, ceftazidime, cefepime and gentamicin (Figure 3). The general prevalence of MDR strains among the three types of hotels was LCH (2.8%), MCH (22%) and 5.2% among HCH hotels.

Figure 3

General Resistance pattern of *E. coli* isolates recovered from healthy food-handlers working in various Hotel-types within Nairobi County on selected antimicrobials



AMP-Ampicillin, **IPM**-Imipenem, **CTX**- Cefotaxime, **CAZ**-Ceftazidime, **ATM**-Aztreonam, **FOX**-Cefoxitin, **FEP**-Cefepime, **AMC**- Amoxicillin-Clavulanic acid, **CIP**-Ciprofloxacin, **NA**-Nalidixic acid, **TE**-Tetracycline, **CN**-Gentamicin, **S**-Streptomycin, **SXT**-Sulfamethoxazole/trimethoprim, and **C**-Chloramphenicol

The Narrow spectrum β -lactamases (NSBLs) phenotype was the most common β -lactamase phenotype (44.6%). The classical ESBL phenotype was only recorded in (3.4%) of the isolates. The inhibitor resistant TEM phenotype (IRT phenotype) characterized by resistance to β -lactamase inhibitors (Amoxicillin-Clavulanic acid) and ampicillin

but with concomitant susceptibility to all other classes of β -lactams was only recorded in 3(<1%) isolates. Advanced β -lactamase phenotypes (pAmpC and CMT) characterized by resistance to majority of cephalosporins and even cephamycins were recorded in less than 2% of the isolates. Table 1-2

Table 1-2
Diversity of β -lactamase phenotypes among the isolates recovered from healthy food handlers working in various food establishments in Nairobi County

Antibiotics to which isolates were resistant							
Penicillin	Third generation cephalosporin or monobactam (e.g Cefotaxime)	Fourth generation cephalosporin (e.g cefepime)	Beta-lactamase inhibitor (AMC)	Cephamycin (e.g. Cefoxitin)	Carbapenem (e.g.imipenen)	Most probable phenotype	Total n(%) of 323
AMP	none	None	-none	-none	- none	NSBL	144(44.6)
AMP	none	-none	AMC	-none	- none	IRT	3(<1)
AMP	CTX ^b /CAZ ^b	-none	-none	-none	- none	ESBL	11(3.4)
AMP	CTX,CAZ,ATM	FEP	AMC	-none	- none	CMT	4(1.2)
AMP	CTX,CAZ	FEP	AMC	FOX	- none	pAmpC	1(<1)

AMP-ampicillin, **CTX**-Cefotaxime, **CAZ**-Ceftazidime, **ATM**-Aztreonam, **FEP**-Cefepime, **AMC**-Amoxicillin-clavulanic acid, **FOX**-Cefoxitin, **NSBLs**-Narrow spectrum β -lactamases, **IRT**-inhibitor resistant TEM phenotype, **ESBL**-Extended Spectrum β -lactamases, **CMTs**- Complex mutant TEMs phenotypes, **pAmpC** -Plasmid-mediated ampicillin β -lactamases phenotypes

b: appearance of zones of synergy between a given cephalosporin and amoxicillin-clavulanic acid(AMC).

None: Isolate that was susceptible to a given class of antibiotics.

As expected, majority (37%) of isolates with the NSBL phenotype tested positive for *bla*_{TEM} but were negative for other advanced classes of β -lactamases such as *bla*_{CTX-M} and the *bla*_{OXA}. In contrast, *bla*_{CTX-M} was the most common gene detected among isolates with the ESBL phenotype (12%). Isolates exhibiting the IRT, CMT and pAmpC phenotypes were few with only one (1) isolate showing the pAmpC phenotype. Other β -lactamase genes such as *bla*_{SHV} and *bla*_{OXA} accounted for 25% and 7% respectively.

The results showed that at 65% similarity level, the isolates analyzed fit into 11 sub-clusters. Isolates from the first two clusters had near homogenous characteristics including resistance phenotypes, type of hotel, gender, education level, clinical and

location of the source participant. All isolates in these two sub-clusters were MDR, 60% of which were obtained from young females (<30years of age) who had a history of self-medication. Majority (80%) of isolates in these two sub-clusters were obtained from participants who had a secondary education. All isolates in these clusters were obtained from people working in middle class hotels. In contrast, isolates making sub-cluster 3-6 were of heterogeneous characteristics such as type of hotel, gender, level of education, location as well as clinical profiles. About 50% of the isolates in these 4 sub-clusters were MDR obtained from males who had a history of self-medicating and working in middle class hotels.

Sub-clusters 7-11 comprised of 86% of isolates from middle class hotels and majority (67%) of these strains were obtained from people who had a tertiary education. About 50% of isolates were from individuals residing within the eastern region of Nairobi commonly characterised by sanitation challenges such as poor sewerage and drainage systems as well as lack of constant potable water to homes and food establishments compared to their counterparts residing in the western regions of the city who have access to almost all social amenities.

DISCUSSION

The significance of emergence and spread of highly MDR strains among food handlers needs no emphasis. Although past studies in Kenya have reported largely on resistance trends among clinical isolates [8][13], few studies have investigated resistance patterns of *E.coli* with special focus on ESBL-producers from food-handlers. Food-handlers who engage in food handling businesses without medical certification or those with poor personal hygiene have shown to increase the possibility of lateral transmission of foodborne infections to their clients as previously reported in studies in Ethiopia, Nigeria and USA [14][15][16].

Globally, self-medication and not completing the required dosage of the prescribed antimicrobial are known key risk factors for emergence and spread of antimicrobial resistant strains [17]. Although self-medication alone may not explain the high resistances observed in this study population, other confounding factors in conjunction with indiscriminate antimicrobial usage may be responsible for the emergence and spread of resistant strains among the

study participants [18][19]. However, imipenem, cefepime and gentamicin were the most potent antimicrobials that can still be used to treat infections arising from these strains. This study further revealed that 3.4% of all isolates were ESBL-producers with about 4.3% of isolates being resistant to amoxicillin-clavulanic acid (a β -lactamase inhibitor) heavily relied upon for treatment of infections caused by ESBL-producers.

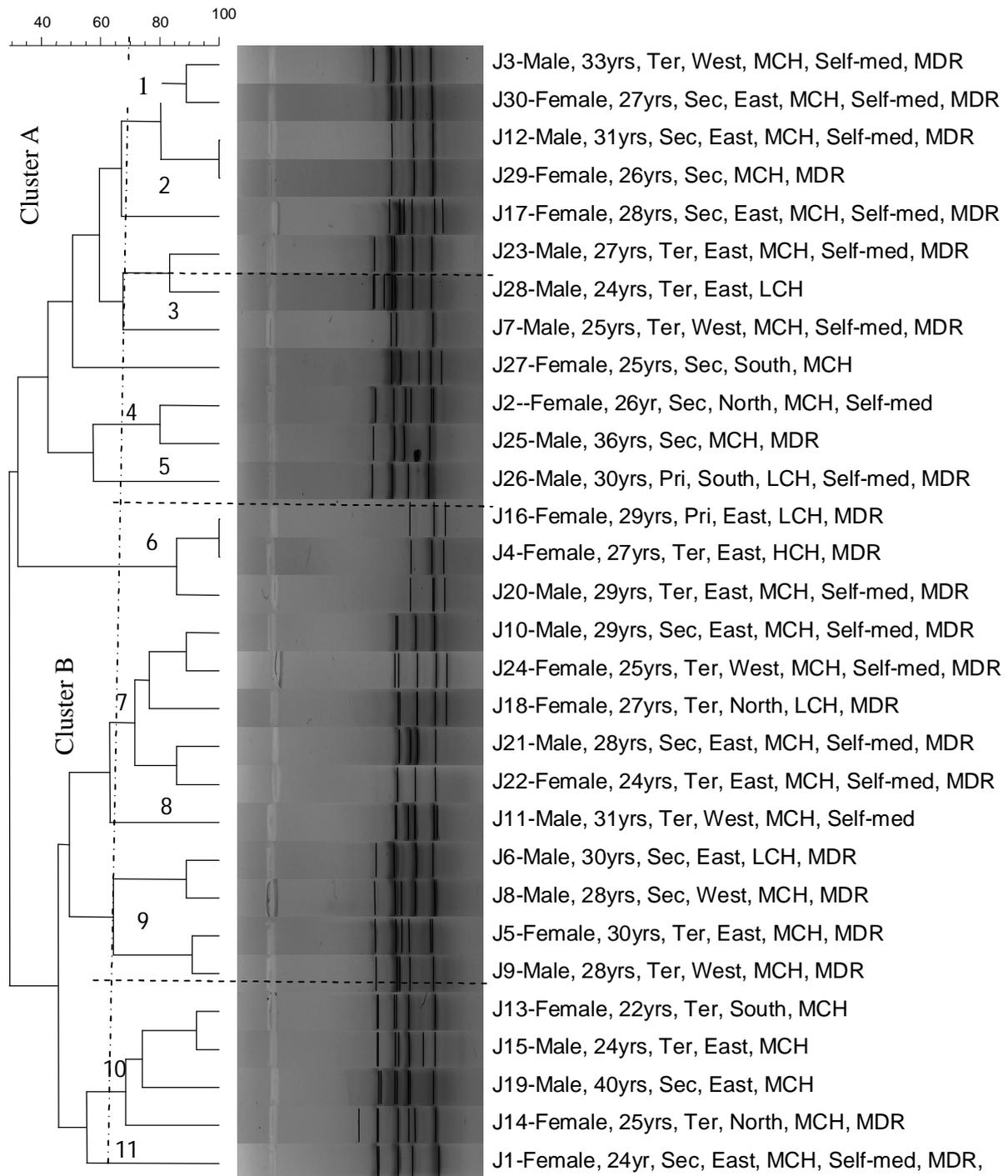
As expected, majority (44.6%) of isolates in this study exhibited the NSBL phenotype, whereas a few (<2%) isolates exhibited the CMT, IRT and pAmpC-phenotypes. These findings vary from those reported among hospitalized and non-hospitalized patients which found that the NSBLs and ESBL phenotypes accounted for 30.5% and 27% respectively, whereas the CMT, IRT and pAmpC accounted for 24%, 8% and 10% respectively [8]. This disparity in findings suggests that clinical isolates are more likely to be more resistant than those obtained from healthy participants probably due to a stronger selective pressure associated with the hospital environment.

Although sequencing of the amplified genes in this study was not done, the CTX-M was found to be the most common ESBL-encoding gene and it's commonly associated with the highly multidrug, rapidly clonally expanding *E. coli* ST131 with resistance towards β -lactams and fluoroquinolones [20][21]. Previous studies in Kenya have reported ST131 strains bearing the *bla*_{CTX-M-15} and/or ciprofloxacin resistance among clinical isolates from hospitalized and non-hospitalized patients [8]. Increase of such resistant strains among food-handlers would lead to spread of these strains to the general public making the containment of these strains difficult.

Genetic analysis using the (GTG)₅ technique suggests that isolates from this study clustered based on medication history and practices of the participants (especially those who self-medicate) and the type of hotel the individual worked in. The study also demonstrated that isolates from middle class hotels are more likely to cluster together indicating close genetic relatedness among isolates from such hotels compared to those from other types of hotels.

Figure 4

Dendrogram showing the clustering of demographic and the resistance profiles of isolates recovered from healthy food handlers working in different food establishments within Nairobi, Kenya



MDR: Multi drug resistant isolates; **Pri:** Primary level of education; **Sec:** Secondary level of education; **Ter:** Tertiary level of education; **LCH:** Low Class Hotels; **MCH:** Middle Class Hotels; **HCH:** High Class Hotel. Geographically the participants working within the capital were conveniently grouped as per their area of residence/locations (East, West, North or South)

CONCLUSION

This study found that a significant proportion of apparently healthy food-handlers do not only carry highly MDR strains but also carry strains that are resistant to classes of antimicrobials that are heavily used for the treatment of infections caused by Gram negative bacteria. Unless measures to control access to and use of antimicrobials in Kenya are put in place, an increase in resistance to such antimicrobials is inevitable. Phylogenetic analysis of isolates obtained in this study further demonstrated that isolates (including the MDR strains) circulating in this apparently healthy population has a potential of spreading to the general public. Considering that foodhandlers, especially those working in MCH come in contact with a significant proportion of people living in urban settings, there is a high likelihood for transmission of resistant strains during an outbreak.

It is therefore important to ensure that proper screening is done before issuance of food handlers' certificate to all food handlers. The hotels' access to better sanitation including access to clean water, toilets and garbage disposal systems should be enhanced. Lastly, it is important to consider vaccinating foodhandlers against some foodborne pathogens such as *Vibrio* and *Salmonella* species that can easily cause large epidemics that can result to high morbidity and/or mortality rates.

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Competing interests

None declared.

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Supplementary Table 1-1
Demographics and resistance profiles of the study participants

Parameters	Variables	Total n (%out of 323)	Non- MDR	n (% of the total per category)			
				MDR n(%)	ESBL n(%)	ESBL+ Cip Resistance combination n(%)	ESBL+CIP+C N Resistance combination n(%)
Gender	Male	165 (51)	120(73)	45(27)	6(3.6)	2(1.2)	1(<1)
	Female	158 (49)	106(67)	52(33)	5(3.2)	0	0
Age	18-30 years	242 (75)	170(70)	72(30)	9(3.7)	1(<1)	0
	>30 years	81 (25)	56(69)	25(31)	2(2.5)	1(1)	1(1)
Marital status	Single	165 (51)	111(67)	54(33)	5(3)	1(<1)	0
	Married	158 (49)	115(73)	43(27)	6(3.8)	1(<1)	1(<1)
Level of Education	Illiterate	1 (0.3)	1(100)	0	0	0	0
	Primary	9 (2.8)	6(67)	3(33)	0	0	0
	Secondary	136 (42.1)	96(71)	40(29)	7(5)	2(1.5)	1(<1)
	Tertiary	177 (54.8)	123(69)	54(31)	4(2)	0	0
Hotel Type	Low end hotel	27 (8)	18(67)	9(33)	0	0	0
	Middle class hotel	245 (76)	174(71)	71(29)	10(4)	2(7.4)	1(<1)
	High end hotel	51 (16)	34(67)	17(33)	1(2)	0	0
Valid license	YES	75 (23)	53(71)	22(29)	4(5)	0	0
	NO	248(77)	173(70)	75(30)	7(3)	2(<1)	1(<1)
Trained in Food hygiene and safety	YES	215(67)	158(73)	57(27)	6(3)	0	0
	NO	108(33)	68(63)	40(37)	5(5)	2(1.9)	1(<1)
Experience(yrs)	< 5yrs	291(90)	199(68)	92(32)	10(3.4)	2(<1)	1(<1)
	>5yrs	32(10)	27(84)	5(16)	1(3)	0	0
Dispensing Point	Health care facility	73(23)	47(64)	26(36)	1(1.4)	1(1.4)	0
	Chemist	246(76)	176(72)	70(28)	9(3.7)	9(3.7)	0
	Others	4(1)	3(75)	1(25)	1(25)	1(25)	1(25)
Type of Medication	Self medication	215(67)	153(71)	62(29)	7(3)	1(<1)	0
	Seek medication	104(32)	70(67)	34(33)	3(2.9)	0	0
	Others	4(1)	3(75)	1(25)	1(25)	1(25)	1(25)
Dosage	Completed	283(88)	195(69)	88(31)	7(2.5)	1(<1)	0
	Incomplete	36(11)	28(78)	8(22)	3(8)	0	0
	Others	4(1)	3(75)	1(25)	1(25)	1(25)	1(25)
Hand washing (after visiting the toilet)	With soap	322(99.7)	226(70)	96(30)	10(3)	2(<1)	1(<1)
	Plain water only	1(0.3)	0	1(100)	1(100)	0	0
Food storage	Fridges	246(76)	176(72)	70(28)	9(3.7)	1(<1)	0
	Container	63(20)	42(67)	21(33)	1(1.6)	0	0
	Others	14(4)	8(57)	6(43)	1(7)	1(7)	1(7)
Waste disposal	Waste bins	305(94)	211(69)	94(31)	10(3)	2(<1)	1(<1)
	Others	18(6)	15(83)	3(17)	1(6)	0	0
Water type	Piped	313(97)	219(70)	94(30)	11(3.5)	2(<1)	1(<1)
	Drilled(Boreholes)	10(3)	7(70)	3(30)	0	0	0
Alternative	Stored in tanks	213(66)	147(69)	66(31)	8(3.8)	2(<1)	1(<1)

water supply	Purchase from vendors	76(24)	56(74)	20(26)	2(2.6)	0	0
	Others	34(10)	23(68)	11(32)	1(2.9)	0	0

The table above shows the summary of the demographic profiles of the participants surveyed with key focus on susceptibility patterns. **CIP**: Ciprofloxacin; **CN**: Gentamicin; **MDR**: multidrug resistant strains; **ESBL+ CIP Resistance**: Strains that exhibited resistance to ciprofloxacin and were also ESBL producers/phenotype. **ESBL+CIP+CN Resistance**: Strains that exhibited ESBL phenotype and also resistant to both ciprofloxacin and gentamicin antimicrobials