International Journal of Endorsing
HEALTH SCIENCE RESEARCH

IJEHSR

Original Article

Antibiogram status of Commensal Strains involved in Multidrug Resistance recovered from famous street foods of Karachi: A threat for public health.

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Doi: 10.29052/IJEHSR.v8.i4.2020.223-230
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Received 14/08/2020
Accepted 13/10/2020
First Published 01/12/2020

OPEN ACCESS

Abstract

Background: Antimicrobial resistance is, as of now, the quintessential issue these days and commensal microorganisms are contributing their part efficiently in disseminating resistance. Thus, this investigation aimed to determine the antibiogram pattern of bacteria recovered from street vended foods in Karachi city.
Methodology: Kirby Bauer's disc diffusion technique was done to check the sensitivity pattern of foodborne bacteria towards 11 standard antibiotics with a range of (11 - 40 mm) inhibition zone.
Results: Nearly all the tested food isolates showed resistance towards oxacillin and amoxicillin. Strains of *S. faecalis* demonstrated 100 % resistance against gentamicin, chloramphenicol, oxacillin, tobramycin, and streptomycin. Ready-to-eat food contaminated with a high bacterial count is an important matter of concern.
Conclusion: Commensal bacteria are responsible for several foodborne sicknesses and are involved in the mechanism of lateral gene transfer. Hence, the exploitation of drugs and violation of acceptable sanitation standards and good hygiene practices must be restricted to restrain the antimicrobial drug resistance and provide good quality food that fulfills the requirements of consumers' wellbeing.

Keywords

Commensal Bacteria, Multidrug Resistance, Street Foods, Commensal Strains, Public Health.

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Introduction

In context with developing countries such as Pakistan, where poverty is the fundamental issue, an enormous amount of readymade foods are processed, made, and consumed by buyers at a cheaper rate[1]. Foodborne diseases are mostly related to microorganisms, such as viruses, bacteria, and parasites. Microbes are the main root of foodborne illness, and their chemicals are released in the form of endotoxins and exotoxins. Cases of hospitalization regarding food poisoning involve bacterial diarrhea and dysentery, while viruses play a minor role, leading to life-threatening severe condition[2].

Clinical manifestation of these foodborne maladies is pretty much alike to other sicknesses. That is the reason several of the foodborne cases are unclear and remain unreported[3,4]. Disturbance in the gastrointestinal tract system is the most common symptom in foodborne cases, including diarrhea, dysentery, retching, nausea, constipation, and stomach spasms. In most cases, it is a mild and self-limiting ailment; thus, no antibiotic treatment is compulsory. However, sometimes the conditions become critical and give a severe and alarming sign to health. Antimicrobial therapy is helpful in severe conditions and for patients that are immunocompromised[5].

The group of Enterobacteriaceae comprises gram-negative microorganisms, facultative anaerobes, non-spore former and, proficient to ferment different types of sugars. Various genera of this family like *Escherichia, Enterobacter, Klebsiella, Proteus, Citrobacter, Serratia, Salmonella, Shigella,* and *Yersinia* are considered potential pathogens. They can be disseminated to water, food, and soil through fecal and wastewater route[6,7].

Most foods derived from animal meat are the primary source of disseminating antimicrobial resistance. Besides, the starting trend of eating and drinking salads and smoothies obtained from plant sources are the center of attention these days and age[8]. These foodborne culprits were accountable for an outburst in the United States for around 1381 individuals got caught with intense gastroenteritis among which 90 % of the deceased cases were reported[9]. One of the chief reasons behind the increase of antimicrobial resistance is an inadequate human practicing and dealing with antimicrobials, such as an incomplete course of antibiotics, unseemly disposing of and flushing of medications, and exploitation of antibiotics, and incorporation of antibiotics in animal's feed[10]. Thus, this investigation's point examination was to check the antibiogram profile of bacteria recuperated from popular street foods of Karachi with an end goal to evaluate the hazard these foods may pose to the public.

Methodology

Bacterial cultures

A total of 54 street food items were microbiologically inspected and 50 bacterial isolates with a percentage dominance including *E. coli* (12 %), *S. fonticola* (10 %), *S. liquefaciens* (2 %), S. aureus (6 %), *C. freundii* (10 %), *L. adecarboxytaca* (2 %), *E.aerogenes* (8 %), *S. faecalis* (2 %), *Pseudomonas spp* (20%), *Bacillus spp* (18 %), *S. epidermidis* (6 %), *S. grimesii* (2 %) and gram-negative rods (10 %) were recouped from famous street vended foods of Karachi city[11].

Antibiotics

The standard antibiotics used were tetracycline (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), amoxicillin (10 µg), levofloxacin (5 µg), streptomycin (10 µg), tobramycin (10 µg), kanamycin (30 µg), and oxacillin (1 µg). These antibiotics were purchased from Thermo Fischer Scientific Oxoid Ltd.

Antimicrobial sensitivity pattern

The antimicrobial sensitivity profile was checked against bacterial isolates by the Kirby Bauer disc diffusion technique and was interpreted by CLSI guideline 2011. The method was proceeded via inoculating tested isolates matched with 0.5 McFarland standard on Mueller Hinton agar plates. Then antimicrobial agent discs were placed on swabbed plates and incubated at 37 °C overnight. After 24 hours, the results were observed in the form of a clear zone of inhibition around the antibiotics discs and measured via meter rule depicted by CLSI. The experiment was performed

in triplicates, and results were shown as mean ± standard deviation.

Results

All the tested street food samples were certain for substantial microbial growth. The antimicrobial sensitivity pattern of bacterial isolates was checked against 11 standard antibiotics ranges between (11-40 mm) as shown in Table 1. All the isolates of E. *coli* and *S. fonticola* demonstrated some susceptibility towards the tested medications except oxacillin and amoxicillin Table 2. Similarly, the same susceptibility pattern was noted against the isolates of *S. aureus, Pseudomonas* species, and *E. aerogenes* in Tables 3 and 4. On the other hand, isolates of *S. fecalis* indicated 100 % resistance against gentamicin, chloramphenicol, oxacillin, tobramycin, and streptomycin Table 4. However, levofloxacin, amoxicillin, and tetracycline have effectively restrained the development of *S. fecalis* isolates. All the five isolates of *C. freundii* were sensitive to seven antibiotics but showed intermediate resistance against levofloxacin and resistance towards oxacillin and amoxicillin. Besides, all the tested gram-negative rods (GNRs) strains were 100 % sensitive to gentamicin, tetracycline, chloramphenicol, tobramycin, streptomycin, and levofloxacin. GNR isolates also showed 80 % vulnerability and 20 % resistance amoxicillin, as shown in Table 5.

Table 1: Antibiogram testing of isolated bacteria from street foods

Antibiotics	Bacterial isolates Inhibition zone measured in millimeter (mm)									
	E. aerogenes	*S.fecalis*	*S. aureus*	*S. fonticola*	*S. grimesii*	*E.coli*	*S. epidermidis*	*C. freundii*	*S. liquefaciens*	*L. adecarboxytaca*
AML	R	31± 0.0	R	R	R	R	R	R	R	R
CN	22 ± 0.0	R	27 ± 0.0	24 ± 0.0	24 ± 0.0	18 ± 0.0	22 ± 0.0	16 ± 0.0	14 ± 0.0	20 ± 0.0
LEV	28 ± 0.0	28 ± 0.0	28 ± 0.0	35 ± 0.0	40 ± 0.0	25 ± 0.0	35 ± 0.0	22 ± 0.0	25.8 ± 0.0	31 ± 0.0
OX	R	R	R	R	R	R	R	R	R	R
C	30 ± 0.0	9 ± 0.0	30 ± 0.0	30 ± 0.0	28 ± 0.0	22 ± 0.0	32 ± 0.0	22 ± 0.0	21.9 ± 0.0	28 ± 0.0
S	19 ± 0.0	R	21 ± 0.0	20 ± 0.0	20 ± 0.0	15 ± 0.0	20 ± 0.0	12 ± 0.0	13 ± 0.0	16 ± 0.0
N	NA	NA	NA	24 ± 0.0	R	10 ± 0.0	16 ± 0.0	NA	NA	11 ± 0.0
TOB	20 ± 0.0	R	28 ± 0.0	21 ± 0.0	NA	NA	NA	20 ± 0.0	19.5 ± 0.0	NA
TET	28 ± 0.0	32 ± 0.0	32 ± 0.0	25 ± 0.0	28 ± 0.0	22 ± 0.0	27 ± 0.0	23 ± 0.0	25.8 ± 0.0	23 ± 0.0
K	NA	NA	24.5±0.7	18 ± 0.0	NA	20 ± 0.0	NA	20 ± 0.0	19 ± 0.0	NA
AK	NA	NA	26.5±0.7	20.5 ± 0	NA	20.5±0.0	NA	21.5±0.7	21.5±0.0	NA

*AML: Amoxicillin, CN: Gentamicin, LEV: Levofloxacin, OX: Oxacillin, C: Chloramphenicol, S: Streptomycin, N: Novobiocin, TOB: Tobramycin, TET: tetracycline, K: Kanamycin, AK: Amikacin, NA: not available, R: resistant

Table 2: Antimicrobial susceptibility profile of *E.coli* and *S.fonticola* isolated from ready-to-eat foods

Antibiotics	Number (%)					
	E.coli (n=6)			*S.fonticola* (n=5)		
	S	I	R	S	I	R
Amoxicillin	0	0	6 (100)	0	0	5 (100)
Gentamicin	6 (100)	0	0	5 (100)	0	0
Tetracycline	6 (100)	0	0	5 (100)	0	0
Chloramphenicol	6 (100)	0	0	5 (100)	0	0
Oxacillin	0	0	6 (100)	0	0	5 (100)
Tobramycin	NA	NA	NA	5 (100)	0	0
Streptomycin	6 (100)	0	0	5 (100)	0	0
Levofloxacin	6 (100)	0	0	5 (100)	0	0
Kanamycin	6 (100)	0	0	5 (100)	0	0
Amikacin	6 (100)	0	0	5 (100)	0	0

*S: Sensitive; I: Intermediate; R: Resistance; NA: not available

Table 3. Antimicrobial susceptibility profile of *E. aerogenes* and *S. faecalis* isolated from ready-to-eat foods

Antibiotics	Number (%)					
	E. aerogenes (n=4)			*S. faecalis* (n=2)		
	S	I	R	S	I	R
Amoxicillin	0	0	4 (100)	2 (100)	0	0
Gentamicin	4 (100)	0	0	0	0	2 (100)
Tetracycline	4 (100)	0	0	2 (100)	0	0
Chloramphenicol	4 (100)	0	0	0	0	2 (100)
Oxacillin	0	0	4 (100)	0	0	2 (100)
Tobramycin	4 (100)	0	0	0	0	2 (100)
Streptomycin	4 (100)	0	0	0	0	2 (100)
Levofloxacin	4 (100)	0	0	2 (100)	0	0
Kanamycin	NA	NA	NA	NA	NA	NA
Amikacin	NA	NA	NA	NA	NA	NA

*S: Sensitive; I: Intermediate; R: Resistance; NA: not available

Table 4: Antimicrobial susceptibility profile of *S. aureus* and *Pseudomonas spp* isolated from ready-to-eat foods

Antibiotics	Number (%)					
	S. aureus (n=3)			*Pseudomonas spp* (n=10)		
	S	I	R	S	I	R
Amoxicillin	0	0	3 (100)	1 (10)	0	9 (90)
Gentamicin	3 (100)	0	0	10 (100)	0	0
Tetracycline	3 (100)	0	0	10 (100)	0	0
Chloramphenicol	3 (100)	0	0	9 (90)	1 (10)	0
Oxacillin	0	0	3 (100)	0	0	10 (100)
Tobramycin	3 (100)	0	0	NA	NA	NA
Streptomycin	3 (100)	0	0	10 (100)	0	0
Levofloxacin	3 (100)	0	0	10 (100)	0	0
Kanamycin	3 (100)	0	0	NA	NA	NA
Amikacin	3 (100)	0	0	NA	NA	NA

*S: Sensitive; I: Intermediate; R: Resistance; NA: not available

Table 5. Antimicrobial susceptibility profile of *C.freundii* and gram-negative rods isolated from ready-to-eat foods

Antibiotics	Number (%)					
	C. freundii (n=5)			GNR (n=5)		
	S	I	R	S	I	R
Amoxicillin	0	0	5 (100)	4 (80)	0	1 (20)
Gentamicin	5 (100)	0	0	5 (100)	0	0
Tetracycline	5 (100)	0	0	5 (100)	0	0
Chloramphenicol	5 (100)	0	0	5 (100)	0	0
Oxacillin	0	0	5 (100)	0	0	5 (100)
Tobramycin	5 (100)	0	0	5 (100)	0	0
Streptomycin	5 (100)	0	0	5 (100)	0	0
Levofloxacin	0	5 (100)	0	5 (100)	0	0

Kanamycin	5 (100)	0	0	NA	NA	NA
Amikacin	5 (100)	0	0	NA	NA	NA

*S: Sensitive; I: Intermediate; R: Resistance; GNR: gram-negative rods; NA: not available

Discussion

Tested bacteria are considered as MDR, demonstrating resistance towards any three tested antibiotics. This can prompt severe precarious conditions for the public as these MDR strains could cause foodborne infections in humans through debased food ingestion. A few discrepancies in the results might be due to the source of bacteria isolated. Consequently, they adopt behavior according to the niche to respond against antibiotics or different chemicals[10]. Multidrug resistance in these isolated organisms could induce selective pressure and actuate the transmission of virulent genes via lateral gene transfer mechanism between and among the strains like *Listeria, Streptococcus, Enterococcus,* and *Staphylococcus*[12,13]. Drug resistance augmentation in the bacteria belonging to the Enterobacteriaceae family is responsible for several fatal diseases in people and animals and should require legitimate vigilance and control over the spreading of these gram-negative microbes. Noteworthy is that the commensal microbes are by and large innocuous for an immunocompetent individual and cause illness in an immunocompromised patient. They can interchange their genetic materials or components (resistant genes on plasmid and chromosomes) with one bacterium to another[14]. The researchers' primary focus is the foodborne pathogens like *Salmonella, Escherichia,* and *Shigella* species, but these commensal bacteria cannot be neglected. In this research, with pathogenic isolate like *Staphylococcus aureus*, other commensal species of *Serratia, Citrobacter, Escherichia,* and *Enterococcus* were recouped.

Citrobacter freundii are responsible for causing many diseases in humans and were considered plausible pathogens. *Citrobacter freundii* are involved in numerous cases of septicemia, bacteremia, respiratory tract infection, and gastrointestinal problems[15]. Only limited information concerning the significance of *Citrobacter freundii* is available[16]. *Citrobacter freundii* are mostly considered as environmental bacteria having a broad range of virulence factors. *Citrobacter freundii* has a likeness with Escherichia. coli as both belong to the same family. Accordingly, *Citrobacter freundii* could prove to be the good beneficiary strain for horizontal gene transfer[17-20]. It is deserving of note that the enteric strains other than E. coli might be clinically and epidemiologically linked with verotoxinogenicity. One outbreak was mentioned in a study that the verotoxigenic *Citrobacter freundii* is responsible for causing serious gastroenteritis followed by hemolytic uraemic syndrome and thrombotic thrombocytopenic purpura by consuming green butter parsley sandwiches[20]. Genus Serratia was formerly considered as a harmless genus to cause rare diseases in humans[21]. Various species of Serratia are involved in histamine-producing foodborne poisoning[22]. Many enteric family members are associated with the enzyme. Histidine decarboxylase is responsible for producing histamine including *Serratia fonticola, Serratia grimesii, Serratia liquefaciens, Proteus mirabilis, Proteus vulgaris, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella spp, and Citrobacter freundii*[23-26].

It was recommended by DeFrancesco et al., 2004 that the commensal bacteria can be utilized for the survey reason to check the predominance of antimicrobial-resistant bacteria. The examination showed that the commensal *Escherichia coli* have a higher rate of resistance against antibiotics than the comparative multitude of MDR *Salmonella* species[27]. The reason for the evolvement of MDR bacteria is the misuse of a wide range of broad-spectrum antibiotics. Through mutation, selection,

and spreading of resistant genes, their occurrences have been expanded[28]. Extended-spectrum β-Lactamase producing enteric bacteria have been isolated worldwide. Their isolation was not particularly restricted to hospital settings but rather from other ecological sources, including food, soil, sludge, healthy humans, animals, and wastewater[29-31]. This is a disturbing sign to deal with; therefore, the condition must be amended before it is excessively troublesome, making it impossible to treat the infection. For this, the European community recommended the control and patrolling measures for these MDR strains[32].

Conclusion

The higher rate of resistance against oxacillin and amoxicillin demonstrated the presence of β - lactamase enzyme in Enterobacteriaceae's family, which is a primary mechanism of antibiotic resistance. All the tested food isolates showed some level of sensitivity towards antibiotics, but all of them were resistant to oxacillin and amoxicillin. While *Streptococcus fecalis* were considered as multidrug-resistant strains showing resistance against 5 antibiotics. These multidrug resistance strains are transferred to humans by consuming water, food, soil, and dust. Such environmental sources expand the rate of persistence of resistant bacteria in public for an extended period. Henceforth, the street foods contaminated with high heaps of microbes that can cause foodborne maladies and exchange resistant genes between and among the pathogenic strains are serious problems and pose a significant health risk for the public.

Conflicts of Interest

None.

Acknowledgement

The author desires to show gratitude to the Food Safety and Research Group team for their support and the FSRG lab to provide the research workplace.

Funding

None.

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