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Occurrence of Multi-drug Resistant Bacteria in Some Selected Street Food Samples

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Antimicrobial resistance is a subject of great concern in the public health. The prevalence of antimicrobial resistance among food pathogens has increased during recent decades.
Studying the incidence and antibiotic resistance pattern of bacterial species isolated from fish and vended street fruits.
Eleven fish swabs and thirteen sliced fruit samples were collected and prepared for isolation of bacterial species through inoculation onto selective and non-selective nutrient media. The grown colonies were purified through subculturing on nutrient agar plates then identified by morphological and biochemical methods. The obtained pure cultures were then kept on nutrient agar slants. Testing antibiotic resistance of the isolated bacterial strains was studied by Kirby-Bauer disk diffusion method on Mueller Hinton agar using ten antibiotics belonging to different classes. The resultant inhibition zone was interpreted according to Clinical Laboratory Standard Institute.
Twenty-eight bacterial cultures were isolated from the collected food samples. The conventional identification using morphological and biochemical methods of these cultures revealed presence of three Gram positive species; *Staphylococcus aureus*, *Streptococcus* sp. and *Bacillus subtilis* in addition to four Gram negative; *Escherichia coli*, *Brucella* sp., *Enterococcus faecalis* and *Proteus mirabilis*. The incidence of the obtained bacterial species was arranged as 29.16% for both *S.*

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aureus and *E. faecalis* followed by *Brucella* sp. 16.66%; *B. subtilis* & *E. coli* 12.5% then *Streptococcus* sp. and *P. mirabilis* with an incidence of 8.33% each. Testing antibiotic resistance pattern of seven bacterial species against ten antibiotics showed that, among three Gram positive bacterial species, only one (33.33%) strain *S. aureus* exhibited resistance to six antibiotics; amoxicillin, erythromycin, ciprofloxacin, ceftriaxone, fluconazole and dicloxacillin. Among four Gram negative bacterial strains only one (25.0%) strain *Enterococcus faecalis* exhibited resistance to eight antibiotics; amoxicillin, streptomycin, chloramphenicol, cotrimoxazole, ciprofloxacin, ofloxacin, sparfloxacin and cloxacillin.

Occurrence of multi-drug resistant bacteria in fish and vended street fruits poses not only risk of disease to the foods but public health hazard to food handlers and consumers in general. Also the result of this study recommended augmentin and cephazolin as good choice antibiotics for treatment of infection in the study area.

Keywords: Antibiotic-resistance; disc diffusion; fish; vended street fruits; Rafha.

1. INTRODUCTION

Antimicrobial agents have saved the human race from a lot of suffering due to infectious disease burden. Without antimicrobial agents, millions of people would have succumbed to infectious diseases. Man has survived the accidental wrath of microorganisms using antimicrobial agents and other mechanisms that keep them at bay. Hardly years after the discovery and use of the first antibiotics was observation made of organisms that still survived the effects of the antimicrobial agents [1].

Every year tons of antibiotic residues are discarded into natural resources, making the environment a reservoir of bacteria carrying resistance genes [2,3]. Moreover, antibiotic resistance is a natural phenomenon in that the microorganisms from the environment and human pathogens share the same resistivity [4,5,6].

Antibiotic resistance has become a global issue, with 700,000 deaths attributable to multidrug-resistance (MDR) occurring each year. Centers for Disease Control and Prevention (CDC) show rapidly increasing rates of infection due to antibiotic-resistant bacteria [7].

Currently, as a result of arising of those MDR organisms options for treatment with antimicrobial agents are limited, and newly introduced drugs placed on the market are decreasing and because it is quite important problem, many efforts are directed to limit those bacteria from spreading [8]. Infection control represents the first steps towards limiting those resistant bacterial types, secondly comes introduction of new effective antibiotics in the market. In the wake of increasing interest in complementary and alternative medicine, herbal extracts are attracted recently in many countries [9,10,11].

One of the most noted consequences of antibiotic misuse and antibiotic pollution is the increased frequency of bacteria harboring ARGs in different environments (here, antibiotic resistance is defined as any reduction in susceptibility in a bacterial strain compared to the susceptible wild type [12,13]. While early antibiotic treatments showed great promise in treating bacterial infections, leading some research to proclaim the elimination of infectious diseases, antibiotic-resistant bacteria were quickly observed following the application of antibiotics at a larger scale [14,15,16].

Monitoring and surveillance of antibiotic resistant bacteria in animals intended for human consumption is important for the regulation of resistance both in animals and man as well as to detect trends and changes of the resistance pattern [17].

The prevalence of antimicrobial resistance among food pathogens has increased during recent decades [18,19,20,21]. The world health organization on animal health (OIE) recommended the continuous monitoring and surveillance of resistant microorganisms in aquatic animals [22] thereby monitoring the trend and level of resistance in the aquatic environment. The multi drug resistance survey in food animals will help to develop guidelines for the prudent use of antimicrobials [23]. In addition, many countries have records of bacterial resistance rate from animals and their products, the public health impact of the isolates and the antimicrobial susceptibility testing of the isolates in an outbreak [24].

Contamination or cross-contamination of street foods, especially sliced fruits and vegetables are increased by unsanitary processing and preservation methods. The use of dirty utensils, as well as the open display of street food produce encourages sporadic visits by flies, cockroaches, rodents and dusts [25]. Food contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe bacterial infections.

The Arab region is rich in natural resources, including plants, herbs and spices. However, most of these remain unexplored for their biological activities including those used in traditional medicine. With the growing problem of bacterial resistance to major classes of antibiotics, which is associated with many of the healthy and economic problems, the search for alternative treatments from natural resources becomes a pressing issue [26]. According to estimation of the World Health Organization, 80% of developing countries peoples rely on the harvested wild plants for their primary health care [27].

The overall study proposes that regular monitoring of multidrug-resistance and proper characterization and assessment of the antimicrobial resistance determinants among bacteria could prevent the dissemination of antibiotic resistant microorganisms. Therefore, the present study was aimed at determining the isolation, antibiotic resistance pattern of bacteria isolated from street vended fruits and fish markets in Rafha governorate at the Northern Border region, Kingdom of Saudi Arabia.

2. MATERIALS AND METHODS

2.1 Chemicals

The media used were nutrient agar, MacConkey agar for isolation of bacteria, Mueller Hinton agar for antibiotic assay (all from HiMedia Laboratories Pvt. Ltd. India). Antibiotic discs used in this study were of Oxoid.

2.2 Sampling

Eleven (11) swabs from the surface of the fish skin, viscera and over the gills during evisceration collected from two fish markets in the public street of Rafha governorate, in addition to thirteen (13) samples of sliced fruits; pineapples (6), watermelons (4) and tomato (3) were purchased from different fruit vendors in the public street of Rafha governorate, at the Northern Border region, Kingdom of Saudi Arabia. The collected swabs were then immediately covered, each fruit sample was placed separately in sterile polythene bags and transported in ice to the laboratory for processing within one hour of collection.

2.3 Preparation of Samples and Isolation of Bacterial Cultures

One set of the swab stick was soaked in nutrient broth and incubated aerobically at 37°C for 24 h for the growth of microorganisms. For the fruit samples, 10 g of each fruit was sliced and dissolved in a conical flask containing 90ml of sterile distilled water. The prepared suspension was diluted up to 10^{-9}. Each sample of swab and fruit dilution was transferred and inoculated separately on the nutrient agar and MacConkey agar plates for growing of non-fastidious organisms and to differentiate between lactose fermenters and non-lactose fermenters, respectively. Then the streaked nutrient agar and MacConkey agar plates were incubated at 37°C for 24 h for the growth of microorganisms.

2.4 Testing of Antibiotic Susceptibility

2.4.1 Preparation of the bacterial inoculums

The bacterial slants were incubated at 37ºC for overnight. The bacterial suspension (0.5 McFarland) was prepared according to the method of Koneman et al. [28]. Briefly, three to five colonies were dispersed in sterile normal saline then turbidity of the test tube was adjusted to 1.5×10^8 CFU/mL that corresponding to 0.5 McFarland standard. Mueller Hinton agar plates were swabbed from the prepared standardized bacterial suspension, then the plates were dried for 3 to 5 min.

2.4.2 Antibiotics resistance pattern

Testing antibiotic resistance of the isolated bacterial strains was studied by Kirby-Bauer disk diffusion method [29] on Mueller Hinton agar using ten antibiotics included the following discs (µg/disc) for Gram positive bacteria; AMX: Amoxicillin (20), STR: Streptomycin (10), E: Erythromycin (15), P: Pencillin G (10 IU), CPX:

Ciprofloxacin (5), VA: Vancomycin (30), CEF: Ceftriaxone (30), CFZ: Cefazolin (30), FLC: Fluconazole (25) and DCX: Dicloxacillin (10). For Gram negative bacteria; AMX: Amoxicillin (20), STR: Streptomycin (10), CHL: Chloramphenicol (30), COT: Cotrimoxazole (25), CPX: Ciprofloxacin (5), OFX: Ofloxacin (5), AUG: Augmentin (30), SP: Sparfloxacin (5), PEF: Pefloxacin (5) and CXC: Cloxacillin (5). The resultant inhibition zone was interpreted according to Clinical Laboratory Standard Institute [30]. Multidrug resistance was defined as resistance to ≥4 antimicrobials [31].

2.5 Statistical Analysis

All values are expressed as the mean ± standard deviation and $P>0.05$ values were considered to indicate statistically significant differences.

3. RESULTS AND DISCUSSION

3.1 Isolation of Bacterial Cultures

Among the collected twenty-four samples (11 fish swabs and 13 street vended fruits), 33×10^5 cfu/ml were counted on the agar plates. It was noticed that the grown colonies were varied in numbers according to the type of sample. The microbial loads of the tested samples are presented in Table 1. Generally, it was recorded that fish samples have the highest microbial load $6\text{-}11\times10^5$ cfu/ml than fruit samples $2\text{-}4\times10^5$ cfu/ml. The recorded microbial load of fruits samples was in the range obtained by Adesetan et al. [32], Nwachukwu et al. [33], Farzana et al. [34], Oranusi and Oluwafemi [35] who recorded microbial load in fruits in the range of 10^4 - 10^9 cfu/ml.

After subculturing purification of these colonies, a total of 28 different bacterial cultures coded RA – 1 to RA – 28 were isolated and purified on different nutrient media. The conventional identification using morphological and biochemical methods of these strains revealed that presence of three gram positive bacteria namely: *Staphylococcus aureus*, *Streptococcus* sp. and *Bacillus subtilis* while the gram negative bacteria using Analytical Profile Index (API 20E) also reveals the presence of four species of bacteria namely: *Escherichia coli*, *Brucella* sp., *Enterococcus faecalis* and *Proteus mirabilis*.

The incidence and occurrence of bacteria isolates from street vended fruits and fish swab samples were presented in Table 2. The results showed that *S. aureus* and *E. faecalis* have the highest incidence of 29.16% for each followed by *Brucella* sp. 16.66%; *B. subtilis* & *E. coli* 12.5% then *Streptococcus* sp. & *P. mirabilis* with an incidence of 8.33% each.

The isolation of these organisms from fish is supported by the work of Grema et al. [36] where who isolated most of the species obtained in our work but in different country; *S. aureus*, *Streptococcus* sp., *E. coli*, *Proteus* sp. and *Brucella* sp. from fish swabs in Nigeria. Presence of these species is interpreted as some are skin normal microflora [37], some are pathogenic microorganisms causing infections [38] as well as some are found in the intestinal tract of fish [39].

Also our results recorded the highest incidence for both *S. aureus* and *E. faecalis*. Udeze et al. [40] reported that, microorganisms from human origin such as *S. aureus*, *E. coli* and *E. faecalis* found to survive and multiply in the gut and tissues of fish which render fish a potential source of human disease over long periods. Regarding interpretation presence of such bacterial species in the fruit samples is supported by the work of Eni et al. [41] and Jolaoso et al. [42] who isolated *S. aureus, Escherichia coli* from fruits. Daniyan and Ajibo [43] also isolated *S. aureus, S. epidermidis, Bacillus* sp., *E. coli* from

Table 1. Range of microbial load of the collected fish and fruit samples

Samples		Microbial load (10^5 cfu/ml)
Type	Number	
Fish swabs (11)	Tilapia (n=4)	6
	Bori (n=4)	11
	Tuna (n=3)	7
Street fruits (13)	Pineapples (n=6)	3
	Watermelons (n=4)	4
	Tomato (n=3)	2

Table 2. Incidence of bacteria isolates in the tested samples

Samples	Bacterial strains (%)						
	S. aureus	*Streptococcus* sp.	*B. subtilis*	*E. coli*	*Brucella* sp.	*E. faecalis*	*P. mirabilis*
Tilapia (n=4)	2 28.57%	– –	1 14.28%	– –	1 14.28%	1 14.28%	– –
Bori (n=4)	1 14.28%	– –	1 14.28%	1 14.28%	1 14.28%	1 14.28%	1 14.28%
Tuna (n=3)	3 42.85%	1 14.28%	– –	1 14.28%	2 28.57%	2 28.57%	– –
Pineapples (n=6)	– –	– –	– –	– –	– –	1 14.28%	1 14.28%
Watermelons (n=4)	– –	1 14.28%	1 14.28%	– –	– –	1 14.28%	– –
Tomato (n=3)	1 14.28%	– –	– –	1 14.28%	– –	1 14.28%	– –
Total (n=24)	7 29.16%	2 8.33%	3 12.5%	3 12.5%	4 16.66%	7 29.16%	2 8.33%

sliced fruits. This is further supported by the work of Oranusi and Olurunfemi [35] who isolated *Bacillus, S. aureus, E. coli, Enterobacter, Proteus* from vended ready to eat fruits sold in Ota, Ogun State.

3.2 Antibiotic Resistance Pattern

The result of the antibiotic sensitivity of the bacterial isolates against ten antibiotics is presented in Tables 3 and 4. From the data recorded in Table 3 it was clear that among three Gram positive bacterial strains, only one (33.33%) strain *S. aureus* exhibited resistance to six antibiotics; amoxicillin, erythromycin, ciprofloxacin, ceftriaxone, fluconazole and dicloxacillin while it showed an Intermediate reaction to two antibiotics; pencillin & cefazolin and sensitive to another two antibiotics; streptomycin and vancomycin.

The data recorded in Table 4 revealed that among four Gram negative bacterial strains only one (25.0%) strain *Enterococcus faecalis* exhibited resistance to eight antibiotics; amoxicillin, streptomycin, chloramphenicol, cotrimoxazole, ciprofloxacin, ofloxacin, sparfloxacin and cloxacillin while it showed an Intermediate reaction to one antibiotic; pefloxacin and sensitive at another antibiotic augmentin. Multi drug resistance to strains is defined as being resistant to four or more antimicrobial agents [44] but sometimes as low as two antibiotics from different classes [45]. The result of this study revealed the presence of two potent multidrug resistant bacterial strains *S. aureus* & *Enterococcus faecalis* are found in fish and street vended fruits in Rafha governorate. The result of this study recommended augmentin and cephazolin as good choice antibiotics for treatment of infection in the study area.

Table 3. Antibiotic susceptibility pattern of gram positive bacterial isolates

No.	Bacterial isolates	Antibiotic resistance pattern									
		AMX	STR	E	P	CPX	VA	CEF	CFZ	FLC	DCX
1.	*S. aureus*	R	S	R	I	R	S	R	I	R	R
2.	*Streptococcus* sp.	R	I	S	R	I	I	S	S	S	S
3.	*B. subtilis*	R	I	I	S	S	I	S	S	S	S

(R)=Resistance, (I)=Intermediate & (S)=Sensitive.
AMX: Amoxicillin, STR: Streptomycin, E: Erythromycin, P: Pencillin G, CPX: Ciprofloxacin, VA: Vancomycin, CEF: Ceftriaxone, CFZ: Cefazolin, FLC: Fluconazole and DCX: Dicloxacillin

Table 4. Antibiotic susceptibility pattern of gram negative bacteria isolates

No.	Bacterial isolates	Antibiotic resistance pattern									
		AMX	STR	CHL	COT	CPX	OFX	AUG	SP	PEF	CXC
1.	*E. coli*	S	S	S	S	S	I	S	S	I	S
2.	*Brucella* sp.	I	R	R	S	S	S	S	S	S	I
3.	*E. faecalis*	R	R	R	R	R	R	S	R	I	R
4.	*P. mirabilis*	S	S	S	R	S	S	S	S	I	S

(R) = Resistance, (I) = Intermediate & (S) = Sensitive.
AMX: Amoxicillin, STR: Streptomycin, CHL: Chloramphenicol, COT: Cotrimoxazole, CPX: Ciprofloxacin, OFX: Ofloxacin, AUG: Augmentin, SP: Sparfloxacin, PEF: Pefloxacin and CXC: Cloxacillin

The presence of *S. aureus* may be explained by the fact that human beings that are processors or vendors carry this organism on/in several parts of their bodies [46]. Also a finding which is supported by earlier reports of Overdevest et al. [47] that antibiotic resistance in *Enterobacteriaceae* has increased dramatically during the past decade. In addition, these results provide evidence that there is an increased emergence of antibiotic resistance from bacterial isolates of fish and vended street fruits.

There is need for the vendors to practice good hygiene to reduce contamination of street vended fruits and fishes with foodborne pathogens. Consumers should wash fruits with clean water before consumption. Vendors and consumers should be educated on the implication of foodborne pathogens in food.

4. CONCLUSION

The findings in this study emphasize the importance of studying multiple genera of bacteria from different foods as sources of human exposure to antibiotic resistance strains. Therefore, presence of multi-drug resistant bacteria from fish and vended street fruits poses not only risk of disease to the foods but public health hazard to fish handlers and consumers in general.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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