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Determination of bacterial contamination isolated from Sandwiches in Kerman City and their resistance to commonly used antimicrobials

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ABSTRACT

This study was conducted to examine the levels of food borne microbial contamination in hand made sandwich samples and their antibiotic resistance characteristics in Kerman, Iran. A total of 134 samples of three types of sandwiches (Hamburger, sausage and Kaalbas) were purchased from 10 different fast-food restaurants of Kerman and examined for the presence of food-borne pathogens and their susceptibility to commonly used antimicrobials . The results of this study showed that Escherichia coli (40.3%) was the most prevalent food-borne pathogen isolate followed by Staphylococcus aureus (4.5%). Susceptibility of all isolates to a variety of antimicrobial agents was tested, and resistance to Cefazoline, Cefixime, Erythromycin, Amoxicillin and Tetracycline was found in 79.5%, 70.6%, 65.7%, 61.8%, and 54.4% of the isolates, respectively. Ciprofloxacin and gentamicin showed highest sensitivity against food-borne pathogens. Escherichia coli isolated from sandwich samples showed a high resistance rate to commonly used beta- lactams[cefazoline (81.4%), cefixime and amoxicillin(66.7%). All of the isolated bacteria samples were resistant to 3 or more antimicrobials. In summary health professionals should plan the strategies to reduce the spread of antibiotic resistant foodborne pathogens through the food chain, with the aim to control their outbreaks in the community.

Key words: Sandwiches, antimicrobial resistance, Iran.

INTRODUCTION

Food-borne diseases are an important cause of morbidity and mortality worldwide. It is estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year[1]. People's increased traveling and free movement of foodstuffs has increased the risk of contracting food poisonings[2].

Infectious disease along with food can be a source of danger, involving multiple agents, mainly bacterial (Salmonella, Campylobacter, Verotoxin producing Escherichia coli (Ecoli), Listeria...), but also parasitic (Toxoplasma gondii, Cyclospora cayetanensis, Trichinella spp...), and viral (Norovirus, hepatitis A virus), as well as non conventional communicable agents and mycotoxins[2-8]. An outbreak of listeriosis among hospital patients in 2004 in Wales, United Kingdom, was epidemiologically linked to the consumption of contaminated sandwiches[7]. Harakeh et al (2005) reported the isolation of Salmonella and Ecoli isolates from meat-based fast food in Lebanon[9]. 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 [3, 10-12]. There are many reports which shows the prevalence of antimicrobial resistance among food-borne pathogens[2, 4-6, 9, 13, 14].

The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades[15-19]. This increase is attributed to the selection pressure created by using antimicrobials in food-producing animals, in addition to the unregulated use of antibiotics by humans in developing countries [20-25].

There is a growing tendency through the consumption of fast foods (hot or cold ready-to-eat foods), especially among urban(civilized) young people in Iran, which increases the risk of food-borne diseases. Like in many other developing countries, there are a few reports about the microbial contamination and antimicrobial resistance epidemiology of fast food hygiene in Iran. So the objective of this study was to determine the prevalence of aerobic food-borne microbial pathogens and their susceptibility to commonly used antimicrobials in sandwiches commonly sold in the marketplace in Kerman City, Iran.

MATERIALS AND METHODS

A total of 134 samples of three types of sandwiches (Hamburger, sausage and Kaalbas) were purchased from Kerman fast-food restaurants of 10 different zone (13-14 samples from each zone) in Kerman city (1,000km from Tehran in south of Iran) between January 2010 to February 2011 for the isolation and identification of microbial pathogens .Each fast-food restaurant was sampled only once.

Overall, sandwiches consisted of (i) hamburger (processed meat), (ii) sausage sandwich (fried sausage slices), (iii) Kaalbas (an Iranian sandwich made of finely hashed or ground, heat-cured beef and lamb sausage and delicately flavored with spices, including ground black pepper, garlic, and whole or ground pistachios, Also it is called Mortadella), lettuce, tomato, and sauces (mayonnaise or ketchup) on a bread roll. Some minor ingredients such as chips, and type of vegetables varied between restaurants depending on the recipe as well as within restaurants based on availability. Sandwich samples were chopped and milled in a commercial blender and then 10 grams of each sandwich sample was separately homogenized in 100 ml of distilled water for 5 min using a mixer.

From this sample 100 µL were streaked on two plates that consist of blood agar supplemented with 5% defibrinated sheep blood and Eosin Methylene Blue (EMB) agar. Plates were incubated aerobically at 37°C for 48 h. Based on colony morphology, positive cultures were Gram stained and Gram-positive organisms were subcultured on blood agar plates (Padtan Teb Co, IRAN) Gram negative rods were subcultured on MacConkey agar (Padtan Teb Co, IRAN). Organisms were identified using standard methods and API Identification System. Gram positive and gram-negative isolates bacterial sensitivity to commonly used antimicrobials [Ciprofloxacin(CIP) , Gentamicin (GEN), Sulfamethoxazole-Trimethoprim (SXT), Erythromycin(ERY), Azithromycin(AZIT), Cefazoline(CEF), Cefixime(CFM), Amoxicillin(AMX) and Tetracycline(TET)) were investigated by disk diffusion method on Mueller-Hinton agar plates using NCCLS guidelines[26, 27].

Data were entered and analyzed by Stata v. 8. and results were reported as the type and percentage of microbial isolations and also the percentage of microbial susceptibility and resistance to commonly used antimicrobials.

Quality control strains Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were included in each run.

Table 1: Type of food-borne pathogen isolates from 134 sandwich samples in Kerman, Iran

Species	Sausages N(%)	Kaalbas N(%)	Hamburger N(%)	All Sources N(%)
E coli	14(10.5)	18(13.4)	22(16.4)	54(40.3)
Staphyl aureus	0	6(4.5)	0	6(4.5)
Klebsiella spp	0	4(3)	0	4(3)
Salmonella	0	4(3)	0	4(3)
Candida albicans	0	2(1.5)	0	2(1.5)
Total Isolates	14(10.5)	34(25.4)	22(16.4)	70(52.3)
No growth	24(17.9)	22(16.4)	18(13.4)	64(47.7)
Total Samples	38(28.4)	56(41.8)	40(29.8)	134(100)

RESULTS

The results of this study showed that culture samples of the 70(52.3%) out of 134 sandwiches were positive, yielding 4 bacteria, one fungi (candida albicans) and 64 samples (47.7%) had no bacterial growth. (Table1).

Escherichia coli (Ecoli) was the most prevalent food-borne pathogen isolate(40.3.%) followed by Staphylococcus aureus (Staph aureus) (4.5%) (Table1). Thirty four out of 56 Kaalbas sandwich samples(63%) showed bacterial contamination followed by hamburger (52.4%) and sausage (36.8%). Table 1 shows the total rate and type of bacterial contamination in three types of sandwich samples.

Table 2 shows the resistance rates of the main isolated pathogens (Ecoli, Staph aureus, Klebsiella spp and salmonella) from sandwich samples to commonly used antimicrobials.

The microbial resistance of Ecoli isolated from sandwich samples to commonly used antimicrobials varied from very low resistance (3.7%) to ciprofloxacin to 81.4% for cefazoline(Table 2).

Staph aureus isolates were highly sensitive to ciprofloxacin and gentamicin100%) but showed high resistance rate (100%) to erythromycin (Table 2).

Table 2: Antimicrobial resistance rates of isolated microorganisms from 134 sandwich samples to commonly used antimicrobials

Species	CIP %	GEN %	SXT %	ERY %	AZIT%	CEF %	CFM %	AMX %	TET %
E coli	3.7	3.8	33.3	55.5	25.9	81.4	70.3	66.7	51.8
Staph aureus	0	0	33.3	100	33.3	33.3	33.3	33.3	66.7
Salmonella	0	0	50	100	100	100	100	0	50
Klebsiella spp	0	**50**	0	100	50	100	100	0	50

CIP, Ciprofloxacin, GEN, Gentamicin, SXT, Sulfamethoxazole-trimethoprim, ERY, Erythromycin , AZIT, Azithromycin, CEF, Cefazoline, CFM, Cefixime, AMX, Amoxicillin, TET, Tetracycline

Salmonella spp and Klebsiella spp isolated from sandwich samples exhibited high levels of antimicrobial resistance (100%) against 3-4 out of the 8 antimicrobials tested (Table 2). Also our results showed the total resistance rate to commonly used antimicrobials was highest for cefazoline (79.5%), cefixime(70.6%) and erythromycin (65.7%) and the lowest resistance rate for ciprofloxacin with(3%) and gentamicin with(4.2%) (Table 3).

Table 3: Frequency of microbial isolates resistance to commonly used antimicrobials from 134 sandwich samples

Antibiotics	Resistant N (%)	Sensitive Intermediate N (%)	Sensitive High N (%)
Ciprofloxacin	4(3.0)	12 (8.8)	118(88.2)
Gentamicin	6(4.2)	8(5.9)	120(89.9)
Trimethoprim -sulfamethoxazole	43(32.3)	8(5.9)	83(61.8)
Erythromycin	88(65.7)	38(28.4)	8(5.9)
Azithromyin	44(32.4)	55(41.2)	35(26.4)
Cefazolin	106(79.5)	16(11.8)	12(8.8)
Cefixime	94(70.6)	16(11.8)	24(17.6)
Amoxicillin	83(61.8)	33(25.0)	18(13.2)
Tetracycline	73(54.4)	51(38.2)	10(7.4)

The prevalence of Ecoli and Staph aureus resistance to more than two or 3 antimicrobials was (96.3%).Salmonella and klebsiella showed complete resistance to 3-4 antimicrobials (data not shown).

Table 3 shows the total frequency of microbial isolates resistance to commonly used antimicrobials. Overall, and among the 8 antimicrobials, the highest rate of resistance among microbial isolates was observed for cefzoline and cefixime and the lowest was for ciprofloxacin and gentamicin.

DISCUSSION

The present study demonstrated that sandwich samples from markets and supermarkets in Kerman City, Iran, were heavily contaminated with *Ecoli* spp. (40.3%). This very high level of contamination indicates a potential breakdown of hygiene at various stages of the food processing and distribution chain and/or a lack of refrigeration of food materials to be used for sandwich preparation. Others also reported the Ecoli as a source of food contamination[9, 18, 28].

Staph aureus was the second most frequent food borne pathogen which was isolated from 4.5% of sandwich samples. Staph aureus is most likely transmitted by hand of food workers[29, 30]. This data is not in agreement with other reports which reported that three pathogens, Salmonella, Listeria, and Toxoplasma, are the most frequent food borne pathogens responsible for 1,500 deaths each year in the United States [1, 5, 29-33].

Salmonella spp was isolated from 4(3%) of samples which is significantly lower than other reports[5, 7, 9, 10, 12, 25].The results showed that sandwich fast foods in Iran could be a public health hazard, as they may act as a potential vehicle for many antimicrobial-resistant pathogenic organisms. Improper hygienic standards and indiscriminate use of antimicrobials are two of the main causes for the prevalence of these pathogenic resistance strains in Iran.

The findings from this study support the need for the health care establishments to follow the recommended guidelines of complete absence of food-borne pathogen isolates in sandwiches at the point of production through an enhanced surveillance system. Also these results emphasize the need to implement protective measures and more emphasis on the application of hygienic practices to reduce the levels of food contamination [1, 31, 34].

Pathogens of fecal, nose or throat, and skin origin are most likely to be transmitted by the hands, highlighting the need for effective hand hygiene and other barriers to pathogen contamination, such as no bare hand contact with ready-to-eat food. The pathogens most likely to be transmitted by food workers are norovirus, hepatitis A virus, Salmonella, Shigella, and Staph aureus. However, other pathogens have been implicated in worker-associated outbreaks or have the potential to be implicated[29, 31].

Improper hygienic standards and indiscriminate use of antimicrobials are two of the main causes for the prevalence of these pathogenic resistance strains in Iran. These results will emphasize the need to implement protective measures and more emphasis will be placed on the application of hygienic practices to reduce the levels of food contamination[1, 31, 34]. It is clear that one overall challenge is the generation and maintenance of constructive dialogue and collaboration between public health, veterinary and food safety experts, bringing together multidisciplinary skills and multi-pathogen expertise. Such collaboration is essential to monitor changing trends in the well-recognized diseases and detect emerging pathogens. It will also be necessary understand the multiple interactions these pathogens have with their environments during transmission along the food chain in order to develop effective prevention and control strategies.[1, 12, 31, 33].

Askarian et al (2004) reported that Iranian food service staff had little knowledge regarding the pathogens that cause food-borne diseases and the correct temperature for the storage of hot or cold ready-to-eat foods, so there is an urgent need for education and increased awareness among food service staff regarding safe food handling practices[35].

E. coli and Salmonella species have marked importance in foodborne diseases and the worldwide emergence of resistant or multi-drug resistant strains of these two bacteria [2, 3, 33].

The microbial resistance of Ecoli isolated from sandwich samples showed a high resistance rate to commonly used beta- lactams[cefazoline(81.4%), and cefixime and amoxicillin(66.7%)] which is clinically important and may indicate inappropriate use of antimicrobials in Iran, however, E. coli showed low resistance to ciprofloxacin (3.7%) and gentamicin (3.8%) which is in agreement with the results of other investigators in other parts of the world [36-38].

Results also indicated that the Salmonella species showed a similar antimicrobial resistance profile to that of E. coli. However, the resistance among Salmonella was higher than that of E. coli. Salmonella species were completely resistant to erythromycin, cefixime and cefazoline.

Although some investigators reported salmonella resistance to quinolones and , penicillins and tetracyclines , however, our data show that resistance to cephalosporins and macrolids (erythromycin and azithromycin) in sandwich Salmonella isolates found in Iran is not in accordance with some other reported values in other countries[5, 25, 39].

Klebsiella pneumonia was relatively resistant to commonly used antimicrobials .Our data are in agreement with several studies in other parts of the world showing an increasing portion of resistant isolates of Klebsiella pneumonia[40, 41].

All of the isolated bacteria samples were resistant to 3 or more antimicrobials.The resistance rates to more than one antimicrobial agent imply that the susceptibility trends of Ecoli and salmonella should be monitored carefully.

Moreover, continuous monitoring should be performed regularly to evaluate levels of contamination with ntimicrobial-resistant bacteria in food production and processing . Since the Ecoli and salmonella resistance could be transmitted to human if animal foods are improperly cooked or otherwise chandelled, so health professionals should plan the strategies to reduce the spread of antibiotic resistant foodborne pathogens through the food chain ,with the aim to control their outbreaks in the community.

Finally, this study may provide a baseline for the contamination status and the presence of antimicrobial-resistant E. coli and Salmonella in the fast foods in Iran. The presence of multi-drug resistant strains is alarming, because such strains lead to a higher fatality rate than sensitive ones. Obviously, the prudent use of antimicrobial agents is a prerequisite for the minimization of the emergence of drug-resistant bacteria, but such prudence in itself is not enough to control this emerging public health threat. Further research and subsequent anagement of this problem is vital to help ensure that the emergence of drug-resistant bacteria is limited and that antimicrobial agents remain effective.

CONCLUSION

Escherichia coli was the most prevalent food-borne pathogen isolate followed by Staphylococcus aureus. Susceptibility of all isolates to a variety of antimicrobial agents was tested, and ciprofloxacin and gentamicin showed highest sensitivity against food-borne pathogens. Escherichia coli isolated from sandwich samples showed a high resistance rate to commonly used beta- lactams (cefazoline , cefixime and amoxicillin)**.** All of the isolated bacteria samples were resistant to 3 or more antimicrobials. So Health professionals should plan the strategies to reduce the spread of antibiotic resistant foodborne pathogens through the food chain, with the aim to control their outbreaks in the community.

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