Original Article

ANTIBIOTIC RESISTANCE PATTERN OF STAPHYLOCOCCUS STRAINS ISOLATED FROM ORANGE AND APPLE JUICES IN SHAHRE-KORD, IRAN

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ABSTRACT

Objective: One of the main routes of transmission of antibiotic resistance in bacteria is through food production. The antibiotics that are used to control diseases are transferred to human through food stuff such as meat, milk, fruit, fruit juices, water and lead to the spread of antibiotic resistant bacteria to human populations. The aim of this study was to determine the pattern of antibiotic resistance of staphylococcus strains isolated from orange and apple juices in Shahre-Kord, Iran.

Methodology: This descriptive-sectional study was carried out on a total of 32 bacterial isolates of *staphylococci* (4 *Staphylococcus aureus* strains and 28 *Staphylococcus epidermidis* strains) isolated from 360 fruit juice samples tested in Shahre-kord. Antibiotic susceptibility test was performed using disc diffusion method and data were analyzed using fishers Z test.

Results: *Staphylococcus aureus* showed 25% resistance to five antibiotics which included tetracycline, co-trimoxazole, amoxicillin, erythromycin and methicillin. *Staphylococcus epidermidis* was the most resistant bacteria to erythromycin. Twenty five percent of the *Staphylococcus aureus* strains and 64.28% of the *Staphylococcus epidermidis* strains were resistant to two or more than two of the antibiotics used in this study.

Conclusions: The results showed that the vast majority of the bacterial isolates were resistant to one or more than one of the antibiotics studied. It is possible for bacterial resistance to result from food products like fruit juices. Therefore it is necessary to restrict the use of antibiotics and control the production, transportation of fruit juices.

KEY WORDS: Antibiotic resistance, *Staphylococcus aureus*, *Staphylococcus epidermidis*, fruit juice.

Pak J Med Sci July - September 2010 Vol. 26 No. 3 615-618

How to cite this article:

Sharafati-chaleshtori R, Sharafati-chaleshtori F, Karimi A. Antibiotic resistance pattern of staphylococcus strains isolated from orange and apple juices in Shahre-kord, Iran. Pak J Med Sci 2010;26(3):615-618

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* Received for Publication: July 20, 2009
* Revision Received: April 5, 2010
* Revision Accepted: April 8, 2010

INTRODUCTION

Microbes (bacteria, fungi, parasites and viruses) cause infectious diseases, and antimicrobial agents (such as penicillin, streptomycin, and over 150 others) have been developed to combat the severity and spread of many of these diseases. The use of antimicrobial agents for preventing or treatment of infection in human, animals and plants, in any dose and over any time period, cause a "selective pressure" on microbial populations. These antibiotic resistant

organisms can then be transferred to humans via consumption of food stuffs such as meat, milk, fruit (fruit juices).[1-5] The incidence of antibiotic resistant bacteria in foodstuff is a major public health threat worldwide.[1] These organisms have been isolated from wide range of foodstuffs consumed by human.[2,6] The development of antimicrobial resistance in many pathogenic microbes poses one of the most serious problems in the control of infectious diseases. Antimicrobial resistance results from the ability of microbes to adapt to anthropogenic pressures; therefore, it is not a passing trend but likely a permanent feature in the fight against infectious diseases.[7] Infections caused by resistant microbes often fail to respond to treatment resulting in prolonged illness and greater risk of death.[8]

Antimicrobial resistance results in increased morbidity, mortality, and costs of health care. In many countries, the high cost of such substitute drugs is prohibitive, with the result that some diseases can no longer be treated in areas where resistance to first-line drugs is widespread. In addition to the cost of drugs, patients infected with resistant microbes often remain sick longer, which increases the costs of health care and is an added financial burden to the family and to society.[9] The inherent advantages of local processing of apple and orange are enormous, reduction of waste through spoilage, and wealth generation. However, the presence of a high microbial load detected in some of the products frequently renders them unfit for human consumption, and therefore unacceptable to the quality conscious markets.[2] In several studies conducted on the resistance of foodborne pathogens, and particularly Staphylococci, most staphylococci giving rise to epidemic infection in hospital today are resistant to a number of antibiotics.[10-13] In the present study, an attempt was made to evaluate the susceptibility pattern of selected strains of *Staphylococcus* strains isolated from the apple and orange fruit products in Shahre-Kord, Iran.

METHODOLOGY

In this descriptive-sectional study, a total of 360 packed apple and orange fruit juices were tested for the *Staphylococci* from August to December, 2006 in Shahre-kord, Iran. The juices were produced by three different local factories in Iran and had at least three-month expiry date at the time of analysis. The fruit juices were serially diluted and 0.2ml of an appropriate dilution was inoculated onto the plate count agar and orange serum plates (QUELAB, UK) in duplicate. The plates were incubated at 37^0C for 24-48 h, and observed for the growth of suspected bacterial colonies. Subsequently, total colony count was determined as previously described.[14,15] Identification and confirmation of the isolates were conducted using the routine bacterial diagnostic methods. They included colonial morphology on blood agar, growth and fermentation on Mannitol salt agar, Gram stain, Catalase, Coagulase and DNase tests. The susceptibility testing of *Staphylococcus* isolates to amoxycillin (amx), 25µg; erythromycin (ery), 15µg; tetracycline (tet), 30 µg; methicillin (met), 5 µg; gentamicin (gen), 10µg; co-trimoxazole (cot), 25 µg; chloramphenicol (chl), 30 µg; and vancomycin (van), 30 µg was carried out by the disk diffusion method as previously described.[16] In brief, the commercial antibiotic discs (HIMEDIA, India) were placed on Muller Hinton agar plates and were incubated at 37^0C for 18-24 hours. Subsequently, the inhibition zones were examined and interpreted as previously described.[17] The potencies of all the antibiotics used in the study were confirmed using susceptible *Staphylococcus aureus* strains (ATCC 25923).

The inhibition zones of ≥20, ≥23, ≥19, ≥14, ≥15, ≥16, ≥18 and ≥15 was considered sensitive for, the antibiotics: amx, ery, tet, met, gen, cot, chl and van, respectively (HIMEDIA, India).

RESULTS

A total of 32 *Staphylococci* (8.8%) were isolated from the 360 fruit juice samples tested with average amounts of 2.3 x 10^3cfu/ml. Four (12.5%) of the isolates were *Staphylococcus aureus* and 28 of them (87.5%) were *Staphylococcus epidermidis*. *Staphylococcus aureus* showed 25% resistance to five antibiotics i.e. tetracycline, co-trimoxazole, amoxicillin, erythromycin and

methicillin. *Staphylococcus epidermidis* showed the most resistance to erythromycin with 57.1%. Twenty five percent of the *Staphylococcus aureus* strains and 64.28% of the *Staphylococcus epidermidis* strains were resistant to two or more than two of the antibiotics used in this study.

Antibacterial susceptibility test was used for *Staphylococcus* strains. *Staphylococcus aureus* strains were resistant to amoxicillin (25%), erythromycin (25%), tetracycline (25%), co-trimoxazole (25%), methicillin (25%) and vancomycin(0%). *Staphylococcus epidermidis* strains were resistant to amoxicillin (28.6%), erythromycin (57.1%), tetracycline (3.6%), methicillin (32.1%), co-trimoxazole (35.7%), chloramphenicol (3.6%) and vancomycin (0%). A high resistance rate was observed among *Staphylococcus epidermidis* isolates, in particular, against erythromycin (57.1%) and co-trimoxazole (35.7%). Thirteen patterns of drug resistance were obtained in the *Staphylococcus* isolates, of which nine were multiple drug resistant (Table-I).

Table-I: Antibiotic susceptibility pattern of the bacterial isolates studied

No of pattern	*Resistance pattern*	*No of isolates*	*Designation*	*%*
1	amx[1]	3	1 St. a*; 2 St. e**	9.3
2	ery[2]	4	1 St. a; 3 St. e	12.5
3	met[3]	3	1 St. a; 2 St. e	9.3
4	chl[4]	1	1 St. e	3.12
5	tet[5], cot[6]	1	1 St. a	3.12
6	amx, ery	2	2 St. e	6.25
7	cot, ery	2	2 St. e	6.25
8	ery, meth	2	2 St. e	6.25
9	amx, cot	1	1 St. e	3.12
10	cot, ery, meth	2	2 St. e	6.25
11	amx, cot ery	1	1 St. e	3.12
12	cot, ery, tet	1	1 St. e	3.12
13	amx, cot, ery, meth	2	2 St. e	6.25

Antibiotic 1-Amoxicillin, 2- Erythromycin, 3- Methicillin, 4-Chloramphenicol 5- Tetracycline, 6- Co-trimoxazole. Bacterial strains: **Staphylococcus. aureus*; ***Staphylococcus. epidermidis* The number of bacterial strains resistant to various antibiotic.

DISCUSSION

This paper provides the local epidemiological data about the antibiotic susceptibility pattern of staphylococcus strains isolated from orange and apple juices in Shahre-Kord, Iran. There is no justification for processed ready–to-eat food being contaminated with these organisms.[18] The processing units of the juices are likely primary causes of high bacterial and fungal load. The maintenance of proper hygienic conditions and use of good quality oranges, apples and water will certainly improve the microbiological quality of these juices, and make them acceptable to quality conscious markets both locally and abroad. As such the establishment of quality control unit/laboratory becomes imperative to detect contamination of either the raw materials or the products early enough. In addition, operators in this sector should utilize the technical assistance of NAFDAC towards attaining acceptable quality standard.[19]

In several earlier works on similar studies carried out on foodstuffs including orange juices, researches have focused mainly on the strains of *E. coli* , *S. aureus, Bacillus cereus and Streptococcus pyogenes*.[20-23] This work, was conducted to evaluate the frequency of antibiotic resistant *Staphylococcus* strains isolated from orange and apple juices. To date, there is no recorded data in our region in this field. However, there are some published results from our region about the prevalence of antibiotic resistant *Staphylococcus* strains isolated from body surface of individuals both in community and hospital settings.[24] The presence of food borne bacterial pathogens such as *Staphylococcus aureus* in the orange and apple juices is considered a safety concern.[25] Since prevalence of antibiotic resistant *Staphylococcus* strains in our region is significant.[24] Antibiotic resistant *Staphylococcus* strains isolated from our work might be related to the other strains mentioned above. *S. aureus* showed 25% resistance to five antibiotics tetracycline, co-trimoxazol, amoxicillin, erythromycin and methicillin. This is similar to with the result of a report indicating the resistance of these strains to the same antibiotics we

used.[2] These results, together, may reflect the widespread use of these antibiotics. The same results were obtained using *Staphylococcus epidermidis.* These strains were resistant to amoxicillin (28.6%), erythromycin (57.1%), tetracycline (3.6%), methicillin (32.1%), co-trimoxazole (35.7%), and chloramphenicol (3.6%).

The relatively high level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment.[2,3,20,23] Antibiotic prescriptions in some hospitals are given without clear evidence of infection or adequate medical indication. Toxic broad-spectrum antibiotics are sometimes given in place of narrow-spectrum drugs as substitute for culture and sensitivity testing, with the consequent risk of dangerous side effects, super infections, and the selection of drug-resistant mutants.[26] The number of such antibiotics ranged from two to seven, and falls within the range obtained by earlier workers.[1,2,11,21] Multiple drug-resistance is an extremely serious public health problem[26] and it has always been associated with outbreak of major epidemics throughout the world.

CONCLUSION

Our results showed that most of the isolates were resistant to one or more antibiotics. It is possible for bacterial resistance to result from food products like fruit juices. Therefore, it is necessary to restrict the use of antibiotics and control the production, transportation and maintenance of fruit juices.

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