ISSN: 2287-6898
International Journal of Bio-Pharma Research
Volume 3, Issue 2 (2014) pp. 182-185
Research Article
Open Access

Multi drug resistance and MAR index among bacteria associated with fruits and vegetables

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Received for publication: Jan 10th 2014; Accepted: Feb 2nd 2014

Abstract: Minimally processed foods particularly fruits and raw vegetables have become popular since it suits the present necessity as need of elaborate preparations are not required. Fruits and vegetables carry microbial flora from the farm or other sources to the table. The present study was aimed at isolation and drug resistance pattern among the organisms isolated from fruits and vegetables. The ability of these isolates to produce biofilms and penicillinase was also assessed. Biofilms help the organisms to survive unfavorable conditions. Total 82.5% of the isolates showed biofilm production on Congo Red Agar medium and 60% showed penicillinase production when tested by filter paper iodometric method. Plasmids among the resistant bacteria may harbor a number of resistance genes and they can be transmitted among the bacteria in environment. These consequences lead to increase in multi-drug resistance. *E. coli, Proteus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Citrobacter* sp., were the prevalent bacteria in fruit and vegetables (intact and unspoiled). Ceftriazone showed maximum resistance in *E. coli* (87.5%) and *Klebsiella* sp. (85.71%). Maximum MAR index of 0.75 was found with *E. coli*.

Key Words: Antimicrobial resistance, Biofilms, MAR index

Introduction

The increase in the number of multi drug resistant bacteria is a major concern of health officials worldwide, particularly with the decline in the number of new antibiotics available for treatment. The number of antimicrobial-resistant (AMR) bacteria in the environment increases exponentially with the use of antimicrobials, which results in increase in selective pressure on bacterial populations. Furthermore, spread of AMR between different bacterial strains in different habitats has been demonstrated. Resistant bacteria from farm animals may contaminate the food produced[1]. Spread of resistance takes place in different ways with respect to clonal spread of resistance strains by the spread of wide host range plasmids and translocatable elements. Commensals in ecosystems have a special significance and a pronounced capacity for acquisition and transfer of resistance genes as with *Enterococcus faecium* and *Escherichia coli* in the gut flora or *Pseudomonas spp*. in aquatic environments[2].

Raw and minimally-processed fruits and vegetables are an essential part of diet all around the world. Fruits and vegetables are widely exposed to microbial contamination[3] through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbor a diverse range of microorganisms including plant and human pathogens. Washing fruits and vegetables with contaminated water and handling of produce by workers, vendors and consumers in the marketplace helps the spread of microorganisms and hence the spread of resistance if such a phenotype dominates in the population of bacteria. Since resistant genes may be in mobile genetic elements the use of specific antibiotic can also induce resistance to other antimicrobial agents and can be transferred to a wide variety of bacteria. Multi drug resistance has been observed in vegetables which are commonly used for salad[4]. The present study was aimed at antibiotic resistance patterns, their ability to produce biofilms and penicillinase production among the bacteria isolated from fruits and vegetables.

Materials and Methods

Sample collection:

Total 75 different types of fruits and vegetables (intact and unspoiled) were collected from Hitech city, Yousufguda, Mehdipatnam, Chintalmeth, Upperpally, Hyderguda areas in Hyderabad in the month of July. All samples were collected in sterile polythene bags and analyzed within four hours after procurement. Samples were rinsed and serially diluted. This was used in streak plate technique on nutrient media for the isolation and identification of strains.

Isolation of gram-negative bacteria:

The media used for isolation and identification of organisms were Mc Conkey Agar (differential media), Eosin-Methylene blue media and Nutrient agar (simple media).

Penicillinase and biofilm production:

Detection of penicillinase production among the isolates was done by filter paper method,

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Sng *et al.*,[5]. Detection of biofilms production was done by using Congo Red Medium, Freemanet *et al.*,[6] Congo Red Agar (CRA) medium was prepared with brain heart infusion broth, sucrose, agar and Congo red indicator.

Morphological and biochemical identification:
Basic bacterial identification methods like Gram staining, IMViC reactions, Catalase, Urease, Oxidase, hydrogen sulphide production were used for the identification of bacteria[7, 8].

Antibiotic sensitivity testing:
Susceptibility of isolates to antibiotics were tested using the disk diffusion Bauer-Kirby Method, against the following commonly used antibiotics using discs obtained from Hi-media, Mumbai. The spectrum of antibiotics tested include β lactamase inhibitor Sulbactum (20mcg), β lactam group- Ceftriazone (30mcg), Cefotaxime (30mcg), Ceptazidime (30mcg) Cefoperazone (75mcg), Cefadroxil (30mcg), Aminoglycosidegroup- Amikacin (AMK:30µg), Netilmicin (NET 30µg), and Gentamicin (GEN 10µg), Ciprofloxacin (CIP 5µg), Sparfloxacin (5mcg), Lomefloxacin (10mcg) that belong to Fluroquinolone group. Discs were consistently tested for efficacy against standard strains recommended by Clinical Laboratory Standards Institute. Inhibition zones sizes were interpreted in accordance to Performance Standards for Antimicrobial Disc Susceptibility Tests, CLSI[9].

Identification of MDR and determining MAR index:
A multiple drug resistance (MDR) phenotype was defined as resistance to 3 or more antimicrobial agents. The multidrug resistance character of the isolates was identified by observing the resistance pattern of the isolates to the antibiotics. Multiple antibiotic resistance (MAR) index, referred to as the number of antibiotics to which test isolate displayed resistance divided by total number of antibiotics to which the test organism was calculated. The MAR index of profile is a good indicator that helps to evaluate the heath risk of the environments for each test isolates by method of Krumperman[10].

Results and Discussion

Out of 75 samples of fruits and vegetables, 50 (66.6%) Gram negative bacteria isolates were identified. Out of these 50 isolates, 30 isolates (60%) were Penicillinase positive. These 30 isolates when tested for biofilm production, 25 isolates were found to be biofilm producers. Morphological, Cultural and biochemical characters were used to identify these 25 isolates[6,7]. For these antibiotic susceptibility tests were done. MDR and MAR index was checked for these isolates. Figure 1, gives the percentage of bacterial isolates obtained. Some of the bacteria isolated in this study may be part of the natural flora of the fruits and vegetables or contaminants from soil, irrigation water, washing/rinsing water or handling by processors and the environment during transportation, the young, the old, the pregnant and the immune compromised consumers potentially have a higher risk of bacterial infection than other groups. This factor is important in risk assessment and risk management relating to the consumption of vegetables[3]. The majorities of bacteria found on the surface of plants were usually Gram-negative and belong to the *Pseudomonas* or *Enterobacteriaceae* groups. Many of these organisms are normally non-pathogenic for humans[11]. Bacteria present vary depending on seasonal and climatic variation. Penicillinase production was found to be 60% among the gram-negative isolates when tested by filter paper iodometric method.

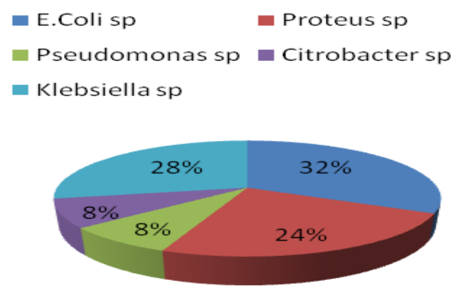

Figure 1: Types of isolates

According to our study, biofilm formation was found to be 82.5%. Biofilms can be found in every environment inhabited by bacteria natural, industrial or clinical media. The presence of biofilms is common in food industry. The attachment of the bacteria to the food product or to contact surfaces leads to serious hygienic problems. Biofilms formed on these surfaces are the main cause of contamination of the final product. The consequences of this contamination are rejection of the product, economic losses and even diseases if food-borne pathogens are involved. In addition to creating problems associated with public health and product spoilage, biofilms are responsible for mechanical blockages and the impedance of heat transfer processes and increase the corrosion rate of surfaces[12]. Table 2 shows the antibiotic resistance pattern observed among the different isolates.

Table 1: Organisms isolated from vegetables and fruits

Types of Vegetables / Fruits	Organisms	Number Of Organisms
Carrot	*Proteus Sp*	1
Spinach	*Pseudomonas Sp*	1
	Klebsiella Sp	2
Sorrel	*Klebsiella Sp*	1
French beans	*Proteus Sp*	1
	E.Coli Sp	1
Amaranthus	*E.Coli Sp*	1
Spring onions	*Pseudomonas Sp*	1
	E.Coli Sp	1
Soya leaves	*E.Coli Sp*	2
Chilli	*E.Coli Sp*	1
	Citrobacter Sp	1
Binjal	*Klebsiella Sp*	2
Local beans	*Proteus Sp*	1
Curry leaves	*Proteus Sp*	1
	E.Coli Sp	1
Tomato	*Klebsiella Sp*	1
Lemon	*E.Coli Sp*	1
Grapes	*Proteus Sp*	2
Peach	*Citrobacter sp*	1
	Klebsiella Sp	1

Table 2: Anibiotic resistant pattern of different isolates

Antibiotics	Conc (mcg)	*E.coli (%) N=8*	*Proteus sp (%) N=6*	*Klebsiella sp (%) N=7*	*Pseudomonas sp (%) N=2*	*Citrobacter sp (%) N=2*
Sulbactum	20	75	100	57.14	100	50
Netilmicin	30	25	16.7	14.2	50	0
Amikacin	75	25	0	28.4	0	50
Gentamicin	10	12.5	0	14.2	50	0
Ceftriazone	30	87.5	33.4	85.71	50	0
Cefotaxime	30	50	50.1	57.14	100	50
Ceptazidime	30	2.5	50.1	99.4	50	50
Cefoperazone	75	75	33.4	71	50	50
Cefadroxil	30	37.5	83.5	71	100	50
Ciprofloxacin	5	12.5	0	28.4	0	0
Sparflioxacin	5	0	16.7	14.2	0	0
Lomefloxacin	10	37.5	16.7	28.4	0	0

In the present study total of 75.8% of isolates were multidrug resistant. Antimicrobial agents are used widely as food additives to improve growth and feed conversion in many types of animal operations, including poultry, swine and cattle operations. As a result, antibiotic resistance in the bacterial communities in the intestinal tracts of domestic animals has become common.

These resistant bacteria may colonise the soil and water bodies, contaminating the vegetable and fruit produce. In that case the natural strain disappears and is replaced by the drug resistant strain. These resistant organisms are responsible for potentially severe infections in the community and have a great capacity for acquisition of resistance to antibacterial agents. Although genes encoding efflux pumps may be present in plasmids, those found in chromosome are often related to the intrinsic resistance mechanisms and enable the bacteria to survive in hostile environments, as for instance in the presence of antimicrobials[13]. Among the major isolates, *Proteus sp* showed maximum resistance with Sulbactum (100%). Ceftriazone showed maximum resistance in *E.coli* (87.5%) and *Klebsiella sp* (85.71%). Maximum resistance seen with Fluoroquinilones and Aminoglycosides was found to be 28.4%.

Table 3: MAR index of different isolates

MAR index	*E.coli*	*Klebsiella sp*	*Proteus sp*	*Citrobacter sp*	*Pseudomonas sp*
0.08	2	0	1	0	0
0.16	0	2	0	1	0
0.33	1	1	3	1	0
0.41	3	1	2	0	1
0.5	0	2	0	0	1
0.58	1	1	0	0	0
0.75	1	0	0	0	0
1	0	0	0	0	0

Table 3 elaborates the MAR index of the different isolates. The Multiple Antibiotic Resistance Index profile is considered as a good indicator that helps to evaluate the heath risk of the environments for each test isolates. MAR index greater that 0.2 implies that the strain of such bacteria originates from an environment where several antibiotics have been used. The MAR index data of isolates showed that majority of the isolates (19 isolates) in the present study are showing MAR index of 0.3 and above. This suggests that a fairly large proportion of the bacterial isolates have been exposed to several antibiotics as the isolates many be nonpathogenic and a part of environment. Maximum MAR index of 0.75 was found with *E. coli*.

Conclusions

This study has shown that the fruits and vegetables were contaminated with bacteria which could be of public health importance. The bacteria were present in various quantities and distributions depending on the fruit or vegetable. Penicillinase production and ability to produce biofilms could enhance the ability of organism to colonies, on the food causing spoilage or in the host if gain entry in to the body. Good sanitary measures should be adopted while handling fruits and vegetables to limit the level of microbial contamination. Identifying the antibiotic resistant bacteria and their resistance pattern will to create awareness among mass people on indiscriminate use of antibiotics.

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Source of support: Nil: **Conflict of interest:** None Declared