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Increasing Threat of ESBL in Raw Vegetables

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Abstract: ***Extended spectrum beta lactamase (ESBL) is an emerging threat for community worldwide since 1963. It spreads through many reservoirs like Hospitals, Sewage, animal/poultry farms to natural water and Food. Agricultural use of antibiotics as growth promoters, use of manure as fertilizer (already contaminated with antibiotic resistant bacteria), and irrigation of vegetable crops with sewage water increases the chances of ESBL presence in raw vegetables. To determine whether ESBL producing gram negatives are present in raw vegetables and in sewage in Surat , we have collected 81 different types of vegetable samples, sewage samples and crop irrigation water samples from different area of Surat and checked for antibiotic resistance using 3rd generation Cephalosporins (Cefotaxime, Ceftazidime). Total of 242 antibiotic resistant isolates found, among them 56.19% from vegetables, 23.14% from sewage and 20.66% from crop irrigation water. Their bacterial species were then analysed for presence of ESBL. 20.68% vegetable samples and 77% sewage samples were found contaminated with ESBL. It shows that raw vegetables irrigated with sewage water may have higher chances to get ESBL. It suggests that raw vegetables might be a source of resistance gene spread among Human.***

Keywords: extended spectrum beta lactamase , Cephalosporins , antibiotic resistant

1. Introduction

Extended spectrum beta lactamases (ESBLs) are enzymes that hydrolyse most beta lactam antibiotics including penicillins and cephalosporins. ESBLs are found in Gram-negative bacteria, especially in *enterobacteriaceae* and *Pseudomonas aeruginosa*.[1,2] The resistance power either spontaneously generated through genetic mutations or by the excessive use of beta lactam antibiotics which gave chance of adaptation to microorganisms. Genetic exchange is another mechanism by which antibiotic resistant plasmids can move between bacteria and transfer resistance capacity. ESBLs are inhibited by clavulanic acid and tazobactam.

About 90% antimicrobial agents used in agriculture as growth promoting and prophylactic agents in animal rather than to treat infection.[3,4] Spraying of crops with antibiotics, particularly fruit trees and vegetables to eliminate surface bacteria is also associated.[4,5] Inappropriate use of antibiotics in the agricultureal setting is a major contributor to the emergence of antibiotic resistant bacteria. This situation was first documented in 1963, when increased levels of resistance in a particular strain of *Salmonella typhimurium* were obsereved at several british feedlots.[6].

A variety of ESBLs, mostly of the CTX-M, TEM and SHV types, have been reported in Enterobacteriaceae.[7,8] TEM was originally found in single strain of E.coli isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM.[9] Being plasmid and transposon mediated has facilitated the spread of TEM to other species of bacteria. The SHV type is most commonly found in K.pneumoniae and is responsible for up to 20% of the plasmid mediated ampicillin resistance in this species.[10] CTX-M preferentially hydrolyze cefotaxime, the serine residue at position 237 plays an important role in the extended spectrum activity of this enzyme.[11]

Animals get antibiotics & develop resistant in their guts. Fertilizers or water contaminating with animal faeces can be used for food crops or meat of that animal which may be not properly cooked might transfers drug resistance to human indirectly. Human faecal carriers discharge into sewages. By these medical & environmental sites get contaminated with ESBLs. If a patient is prone to infection and get infected by ESBLs, it can be more difficult to treat, because many of the commonly used antibiotics will not work against ESBLs. Therefore the aim of this study was to evaluate the presence of ESBL producers in raw vegetables and for that we collected samples of raw vegetables and sewage.

2. Materials & Methods

2.1 Samples

Samples were collected from different area of Surat, Gujarat, India. As raw vegetables we have collected 45 samples. Which is of lettuce, Drumstick, coriander leaves, Fenugreek leaves, Capsicum, sprouts etc which were commercially available from market and organic store and 36 samples of canal, bore well, river, sewage water which used in irrigation for agricultural purpose.

2.2 Screening of isolates

Water Samples were inoculated onto Drigalski lactose selective agar plates containing 2mg/L cefotaxime (Drigalski-CTX), 2mg/L ceftazidime (Drigalski-CAZ) and incubated at 37°c for 24-48h under aerobic conditions. Each vegetable sample (1gm) was placed in 10ml of sterile distilled water and mixed by vortex machine & then one loopfull sample was inoculated onto 2mg/L Drigalski-CTX & Drigalski-CAZ medium separately following the incubation of 24-48h at 37°c under aerobic conditions. All samples were processed within 5 hours of collection.[12]

2.3 Phenotypic confirmation

Confirmation of presence of ESBL producers were carried out by Kirby- bauer disk diffusion method according to CLSI guidelines [13]. They were identified by combination between cefotaxime (CTX) and cefotaxime/clavulanic acid

(CEC) and similarly with Ceftazidime (CAZ) and ceftazidime/clavulanic acid (CAC) discs. The concentration was 30µg of Cefotaxime & Ceftazidime & 10µg of clavulanic acid according to CLSI guidelines. The procedure was followed by inoculating antibiotic resistant isolate from Drigalski medium (turbidity according to 0.5 McFarland) through spreading swab on Mueller-Hinton agar, placing commercially available antibiotic disks with proper distance to each other & incubation of 24- 48h at 37°c under aerobic conditions.

2.4 Bacterial species identification

ESBL producers identified from morphological characteristics of colony, Gram staining reaction, Motility, various biochemical test with reference of Bergey's Manual of Systematic Bacteriology 2005

3. Result

Out of 81 samples collected - 45 of raw vegetables and 36 of water samples, there were growth of gram negative rods as the medium used was selective for isolation of Gram negative bacteria. Except 9.87% of the total sample- 90.12% all showed growth of Antibiotic resistant gram negatives. Percentage ratio of isolates which shows resistance towards ceftazidime, Cefotaxime or towards both is shown in figure1.

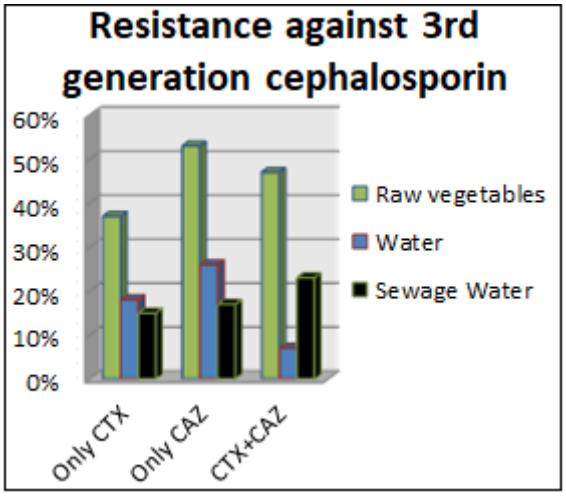

Figure 1: Percentage isolates from different samples that shows resistance against 3rd generation cephalosporins (cefotaxime(CTX), ceftazidime(CAZ))

13.23% of the 3rd generation cephalosporin resistant isolates from raw vegetables were possessing ESBL producers. Which results in 20.68% prevalence of ESBL in raw vegetables. ESBL producers were confirmed by Kirby-bauer disk diffusion method according to CLSI guidelines shown in figure 2.

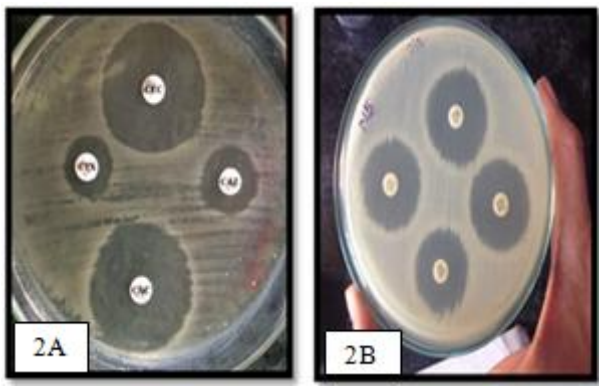

Figure 2: Figure 2A Shows difference of zone size between 3rd generation cephalosporin and its inhibitor (CTX/CEC, CAZ/CAC) which confirms ESBL producer as ESBL producer can't resist the antibiotic in presence of inhibitor. Figure 2B shows no difference of zone size which confirms non ESBL producer according to CLSI and EUCAST guidelines.

From total sewage water samples 56 gram negative 3rd generation cephalosporin resistants were isolated, of which 37.5% were ESBL producers and resulted in 77.77% prevalence of ESBL producers in Sewage. The prevalence of ESBL producers is higher in Sewage compare to water and raw vegetables as shown in figure 3.

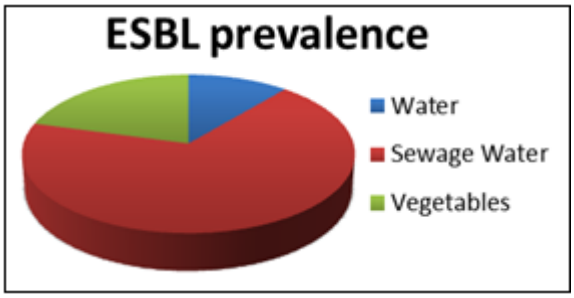

Figure 3: Prevalence of ESBL producers in different samples collected from different area of Surat during study period.

By performing various biochemical tests according to Bergey's Manual of Systematic Bacteriology 2005 it was found that among total isolates 21.66% ESBL producers were from *Escherichia* genus, *31.66%* from *Serratia* genus and others were from *Salmonella*, *Enterobacter*, *Providencia*, *Citrobacter*, *Leminorella*, *Yersinia*, *Kluyvera* and *Cedecea* genus as shown in figure 4.

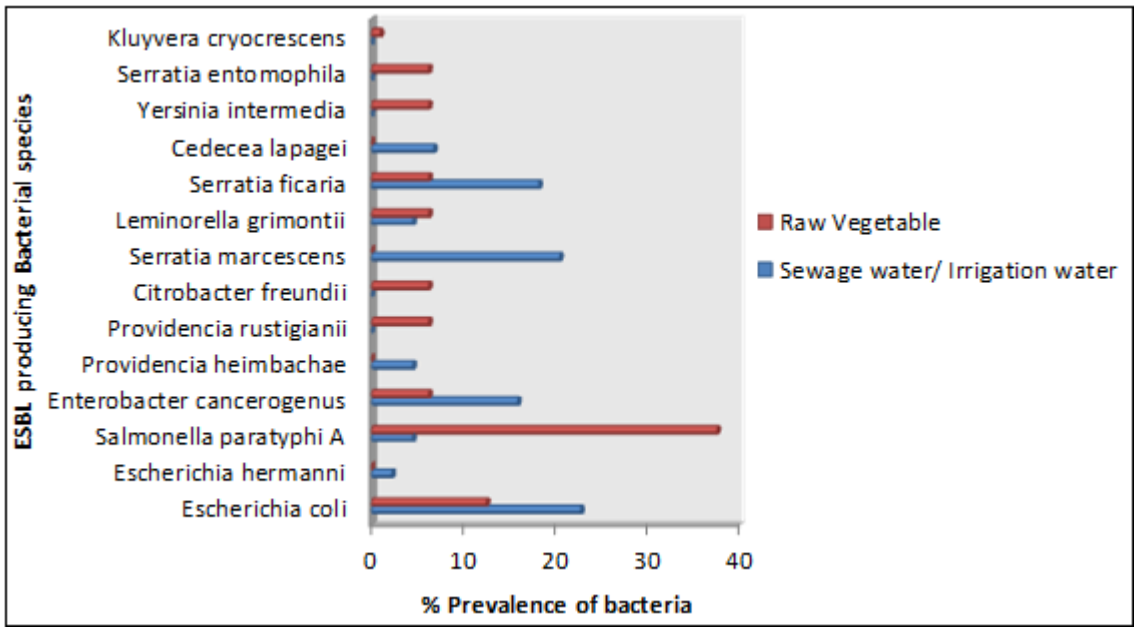

Figure 4: Percentages of Bacterial species isolated from raw vegetable and water samples which were resistant towards 3rd generation cephalosporins- Cefotaxime (CTX), Ceftazidime(CAZ) and also extended spectrum beta-lactamase(ESBL) producers.

4. Discussion

There are studies that indicate vegetables as a possible route for spreading of resistance genes throughout community. [14,15,16,17] Since last 70 years level of antibiotic genes in soil is increasing [18]. Aquatic system and sewage are also reservoirs of antibiotic resistance genes due to antibiotic use and waste disposal [14,19,20]. Fresh produce can also become contaminated during processing. [15]. Because of the use of manure only , no pesticides and a different processing of different microorganisms , contaminants found on organic produce compared to conventionally grown vegetable [21]. We found that many enterobacterial spp. Other than *Escherichia coli* are responsible for antibiotic resistance. Studies suggest that these enterobacterial spp may act as a reservoir of mobile drug resistance genes and transfer them to non-pathogenic *Escherichia coli* present in gut [22,23]. In a study of ESBL producing *Enterobacteriaceae* in different environments, 0.4% foods found to harbor ESBL producing *Escherichia coli* and *Klebsiella pneumonia*. [14]. In 2014 Reuland EA reported about 6% prevalence of ESBL producing Enterobacteriaceae in raw vegetables in Amsterdam [24]. Prevalence of ESBL producing *Enterobacteriaceae* in vegetables imported from Dominican Republic, India, Thailand, and Vietnam was 25.4% [25].

5. Conclusion

From the present study it can be concluded that prevalence of ESBL producing *Enterobacteriaceae* in raw vegetables is increasing and raw vegetables can be a route of emergence of extended spectrum beta-lactamase in community and environment. Elimination of excess use of antibiotics in agriculture, discovery of effective antibiotics for resistant bacteria, monitoring the prevalence of ESBL producers in environment are certain practices which may decrease the threat of ESBL in raw vegetables.

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