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Occurrence and Antimicrobial Resistance of *Enterococcus* spp. Isolated from Lettuce and Irrigation Water in Abidjan, Côte d'Ivoire

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HIGHLIGHTS

- *Enterococcus faecalis* was found in 75% of lettuce and 80.5% of irrigation water samples.
- The most antibiotic-resistance rates in *Enterococcus* isolates were related to erythromycin and co-trimoxazole.
- There is public health concern regarding raw consumption of lettuce in Abidjan city.

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Acronyms and abbreviations
BEA=Bile Esculin Agar
CFU=Colony Forming Unit

ABSTRACT

Background: *Enterococcus* spp., belonging to the group of lactic acid bacteria, are Gram-positive ubiquitous commensals of the intestines of human beings as well as warm-blooded animals. The main objective of this study was to determine the occurrence and antimicrobial resistance of *Enterococcus* spp. isolated from lettuce and irrigation water in Abidjan, Côte d'Ivoire.

Methods: A total of 72 samples, including leaves of lettuce (n=36) and irrigation water (n=36) were randomly collected from three different agricultural sites located in Abidjan city, Côte d'Ivoire. After microbial analysis and identification of *Enterococcus* spp. by culturing and biochemical methods, antimicrobial susceptibility tests were carried out using disk diffusion method. Data were analyzed by statistical processing software R (R 3.0 for Windows).

Results: *E. faecalis* was recognized as the most prevalent strain which was found in 27 out of 36 (75%) lettuce as well as 29 out of 36 (80.5%) irrigation water samples. The mean *Enterococcus* load of lettuces and irrigation water samples were 2.3±0.7 and 3.6±2 log Colony Forming Unit per g lettuce, respectively. Among 45 studied enterococci isolates, the most antibiotic-resistance rates were related to erythromycin (54%) and also co-trimoxazole (49%).

Conclusion: There is a considerable public health concern regarding raw consumption of lettuce cultivated in Abidjan city which can cause gastroenteritis diseases in consumers.

Introduction

Enterococcus spp., belonging to the group of lactic acid bacteria, are Gram-positive ubiquitous commensals of the intestines of human beings as well as warm-blooded animals (Gomes et al., 2008; Murray, 2000; Silva et al., 2011). Although certain enterococcal strains may have desirable functional or probiotic effects on human health (Araújo and Ferreira, 2013; Nueno-Palop and Narbad, 2011; Pieniz et al., 2014), some other species cause human infections such as bacteremia, endocarditis, urinary tract as well as central nervous system infection, etc. These pathogenic species mainly have multiple antibiotic

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resistance as well as some virulence factors (Chajecka-Wierzchowska et al., 2017; Nueno-Palop and Narbad, 2011; Pieniz et al., 2015; Rehaiem et al., 2016). In recent years, the number of antibiotic resistant enterococci has been increased, causing some public health concerns (Koffi-Nevry et al., 2011; Nagulapally, 2007).

Unfortunately, due to poor hygiene practices in agricultural farms in some areas of Abidjan, Côte d'Ivoire, usage of irrigation water contaminated to domestic and hospital effluents in likely and so, the cultured fresh produces such as lettuce have potential risk of contamination to enterococci. The main objective of this study was to determine the occurrence and antimicrobial resistance of *Enterococcus* spp. isolated from lettuce and irrigation water in Abidjan, Côte d'Ivoire.

Materials and methods

Sampling

From March to May 2014, a total of 72 samples, including leaves of lettuce (n=36) and irrigation water (n=36) were randomly collected from three different agricultural sites (Port-Bouet, Adiopodoumé and M'Pouto) located in Abidjan city, Côte d'Ivoire. The collected samples were put into cold boxes, transported to the laboratory for next bacteriological analysis.

Microbial analysis

Total enterococci counts were performed according to the method described by Vanderzant and Splittstoesser (1992). A culture was prepared on Bile Esculin Agar (BEA) medium (Biorad, Marne-la-coquette, France) without antibiotic and on the same medium supplemented with 6 mg/L of vancomycin, which selects enterococci resistant to this antibiotic. The incubation was performed at 37 °C for 24 h. The role of the vancomycin in agar plate consisted to isolate and screen a multi-drug resistant strain such as vancomycin-resistant enterococci among the total flora of the enterococci. The small translucent colonies with halo (black halo), having characters of cocci, Gram-positive, and catalase-negative were taken into account for enumeration of enterococci total flora. Results were expressed as a Colony Forming Unit per g lettuce (CFU/g) or CFU per ml (CFU/ml) of the analyzed water. The identification of *Enterococcus* species was carried out from presumptive colonies obtained on BEA using the biochemical test of the fermentable sugars in particular, arabinose, mannitol, sucrose, sorbitol, and the potassium tellurite tests.

Antimicrobial susceptibility tests were carried out using disk diffusion method as described by Faour-Klingbeil et al. (2016). The susceptibility testing was carried out by culturing strains on Mueller-Hinton agar (Bio-rad, Marne-la- coquette, France). The antibiotic disks (Bio-rad, Marne-la-coquette, France) were used in this research consisted as follow: vancomycin, 30 µg; ampicillin, 10 µg; erythromycin,15 µg; teicoplanin, 30 µg; rifampicin, 30 µg; streptomycin, 500 µg; gentamicin, 500 µg; chloramphenicol, 30 µg; pristinamycin, 15 µg; as well as co-trimoxazole, 1.25 µg. It should be indicated that the reference strain *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as positive-control for the evaluation of the medium BEA.

Statistical analysis

The statistical processing software R (R 3.0 for Windows) was used for the exploitation of the results of the average loads, expressed in CFU/g or CFU/ml. The difference between variables was considered significant at $p<0.05$.

Results

All the lettuce and irrigation water samples were contaminated to *Enterococcus* spp. *E. faecalis* was recognized as the most prevalent strain which was found in 27 out of 36 (75%) lettuces as well as 29 out of 36 (80.5%) irrigation water samples and the remain samples were contaminated to the other species including, *E. faecium*, *E. gallinarum*, *E. casseliflavus*, and *E. durans*. The mean *Enterococcus* load of lettuces and irrigation water samples were 2.3±0.7 and 3.6±2 log (CFU/g), respectively. No significant difference ($p>0.05$) was found between the contamination levels of lettuce samples and their origin site as shown in Figure 1. Similarly, there was not any meaningful relation ($p>0.05$) between the contamination levels of irrigation water samples and their origin site which is illustrated in Figure 2.

Among 45 studied enterococci isolates, the most antibiotic-resistance rates were related to erythromycin (54%) and co-trimoxazole (49%) as shown in Figure 3. There was not any significant difference between rates of antibiotic resistance in strains pertaining to lettuce and irrigation water samples ($p>0.05$).

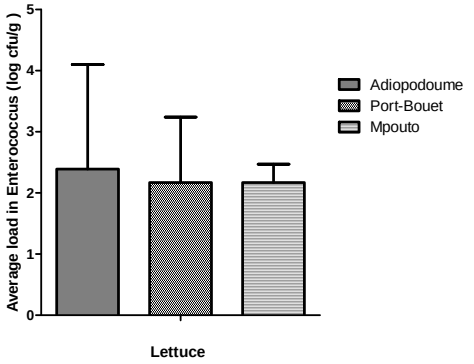

Figure 1: Average load of *Enterococcus* spp. (log CFU/g) in lettuce samples based on their origin site

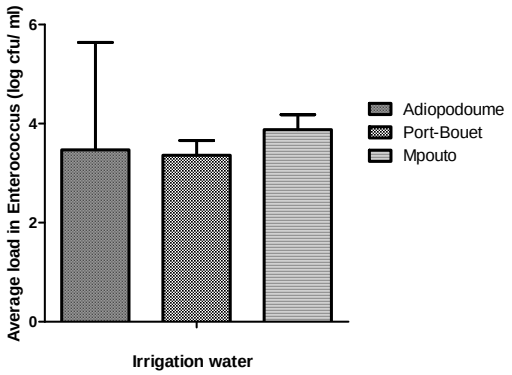

Figure 2: Average load of *Enterococcus* spp. (log CFU/g) in irrigation water samples based on their origin site

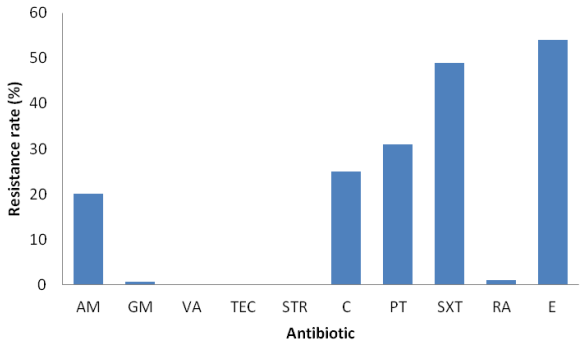

Figure 3: Resistance rate (%) of *Enterococcus* isolates for various antibiotics (AM: ampicillin; GM: gentamycin; VA: vancomycin; TEC: teicoplanin; STR: streptomycin; C: chloramphenicol; PT: pristinamycin; SXT: co-trimoxazole; RA: rifampicin; E: erythromycin)

Discussion

The current study showed a significant presence of fecal enterococci especially *E. faecalis* in the lettuce and irrigation water samples in Abidjan area. Contamination of lettuce by these faecal enterococci would come from shallow water sources contaminated to faeces of livestock and human which are used for irrigation of farm lands (Holvoet et al., 2013). Consideration concurrent contamination of both water and lettuce samples seen in this study, it seems probably that the water intended for the irrigation of lettuces grown on the lagoon bank of the Abidjan city is a source of direct contamination by antibiotic resistant enterococci. Such high rate of contamination with *E. faecalis* detected in water samples of this study was similar to those described by Nagulapally (2007). However, other pathogenic bacterial genera such as *Listeria* and *Escherichia* have been detected in lettuce samples irrigated with contaminated water which were respectively reported by Oliveira et al. (2011) and Njage and Buys (2015). Another study by Njage and Buys (2017) showed a very high contamination rate of lettuces by *E. coli* originated from irrigation water. So, there is a close relation between contamination rate of irrigation water and harvested crops indicating the role of water as the vector of contamination of fecal bacteria in the farm lands. On the other hand, Van Der Linden et al. (2014) showed that the survival of enteric bacteria in lettuces depended on the temperature and chemical characteristics (e.g. salinity) of irrigation water.

Concerning antibiotic susceptibility of *Enterococcus* spp., our results showed that the highest resistance rate was related to erythromycin. In comparison, according to Holvoet et al. (2013), the highest antibiotic resistance rate in *E. coli* isolated from lettuce, irrigation water, and soil in Belgium was found for ampicillin, followed by cephalothin, amoxicillin-clavulanic acid, and tetracycline. Another research revealed that all the *E. coli* isolates from raw vegetables in Lebanon were resistant to streptomycin (Faour-Klingbeil et al., 2016). The diversity of antibiotic patterns seen in the previous investigations could be due to several factors such as geographical area of sampling and hygienic condition of agriculture.

Conclusion

There is a considerable public health concern regarding to raw consumption of lettuce cultivated in Abidjan city which can cause gastroenteritis diseases in consumers. In order to prevent the spread of these multi-resistant enterococci in the food chain in the future, preventive measures should be taken as soon as possible by local authorities.

Conflicts of interest

All the authors indicated that there is no conflict of interest in this research.

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