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.

**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-
Wierczowska et al., 2017; Nueno-Palop and Narbad,
2011; Pieniz et al., 2015; Rehaim 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 agricul-
tural 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; gentami-
cin, 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
Average load in Enterococcus (log cfu/ ml)

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 et al., 2013). This work was financed by the Pasteur Institute of Côte d’Ivoire. The research was carried out with scientific collaboration of Nangui Abrogoua University and Pasteur Institute of Côte d’Ivoire. The authors thank Mrs. Nathalie Guessennd (Head of local institutional review board).

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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