# USE OF CHROMAGAR ORIENTATION FOR PRESEMPTIVE IDENTIFICATION OF ENTEROCOCCI AND CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE OF THE ISOLATES

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**Abstract:** This study was conducted to evaluate the use of CHROMagar Orientation for presumptive identification of enterococci from poultry, and to characterize the antimicrobial resistance of the isolates. Strains identification with conventional methods allowed confirmation of *Enterococcus* genus membership of all of the isolates on CHROMagar orientation (100%). Of the 141 enterococci isolates obtained from chicken, 81 (57.45%) were identified as *Enterococcus faecalis* and 60 (42.55%) as *Enterococcus faecium*. The antimicrobial susceptibility test presented high level of resistance to Tetracycline (89%) and Erythromycin (65%), low level of resistance to High Level of Streptomycin, Penicillin and Ciprofloxacin (13%, 10% and 9% respectively). Few of isolates were resistant to Chloramphenicol (2%). All the strains were susceptible to High Level of Gentamycin, Ampicillin, Vancomycin and Nitrofurantoin. The predominant phenotype of resistance pattern identified in both *E.faecalis* and *E.faecium* was (Erythromycin -Tetracycline).

**Key words:** Antibiotic resistance, CHROMagar Orientation, *Enterococcus*, Poultry.

## INTRODUCTION

Enterococci are natural inhabitants of the human and animal gastro intestinal tract. Their role in opportunistic and nosocomial infections has increased significantly in recent years. Added to this, they have the ability to acquire genes of resistance to several antibiotics, which compromise the choice of therapy. Hence the need for rapid identification of enterococci. CHROMagar Orientation claims to facilitate and expedite the identification of commonly isolated gram-negative bacteria and some gram-positive bacteria such as *Enterococcus spp*. without confirmatory testing, on the basis of different contrasted colony colors produced by reactions of genus or species specific enzymes with a proprietary chromogenic substrate Merlino et al. (1996).

The aim of this study was to evaluate the use of CHROMagar Orientation for isolation of enterococci from poultry and to characterize the antimicrobial resistance of the isolates.

## MATERIAL AND METHODS

**Sample Collection:** Samples were collected once each week from four poultry slaughterhouses located in the Wilaya of Tizi Ouzou, Algeria, as previously described (Yousfi and Bachir Pacha, 2018), between January 2018 to June 2018.

**Strain isolation:** CHROMagar Orientation (Becton Dickinson) was used for presumptive identification of *Enterococcus spp*. The principle of this medium is the use of chromogenic substrates revealing metabolic enzymes.

After enrichment of the specimens on Brain Heart infusion broth supplemented with 6.5% of NaCl for 48h at 37°C, one typical enterococcal colony growing on CHROMagar orientation plates, at 37°C for 24h (Small blue-green colonies), was selected and subcultured on Columbia agar (Oxoid) with 5% sheep blood for purification, from which the isolates were selected for further identification and characterisation.

The appurtenances to the genus level of the isolates were confirmed by positive Gram staining, the absence of catalase and growth on Bile-Esculine Agar with esculine hydrolysis (Facklam and Colinsm, 1989).

**Species identification:** *E.faecalis* is distinguished from *E.faecium* and other species by its ability to grow in media containing 0.04 % tellurite, (B.E. Murray, 1990). Identification of the others species was carried out by API 20 Strep test kit (BioMerieux), according to the manufacturer's guidelines.

**Antimicrobial susceptibility test:** Antimicrobial profile of tested isolates of *Enterococcus spp*. was evaluated using the qualitative disk diffusion method on Mueller-Hinton agar medium (IPA), according to the recommended Clinical Laboratory Standards Institute guidelines, (CLSI, 2017).

Ten antibimicrobial agents were tested, the antibiotic concentration per disk (Oxoid) was as follows: Tetracycline (30 µg), Erythromycin (15 µg), High Level Streptomycin (300 µg), High Level Gentamycin (120 µg), Ampicillin (10 µg), Penicillin (10 UI), Vancomycin (30 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Nitrofurantoin (300 µg).

The diameters of inhibition zones were interpreted by referring to the table of *Enterococcus spp* as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2017), and the isolates were categorized as susceptible, intermediate or resistant (S, I or R).

*Staphylococcus aureus* ATCC® 25923 and *Enterococcus faecalis* ATCC® 29212 obtained from American Type Culture Collection were used as quality control organisms.

The Chi-square test was used to determine the significance of differences in antibiotic resistance rates among isolates, where appropriate. A P value less than 0.05 was considered statistically significant.

## RESULTS AND DISCUSSIONS

**Occurrence of *Enterococcus spp*.:** A total of 47 flocks were sampled, and 141 pools were created. Of the 141 samples analyzed for the presence of enterococci, 100% of them were positive. The species identified were: *Enterococcus faecalis* with a rate of 57.45% (81), and *Enterococcus faecium* 42.55% (60).

**Antibiotic susceptibility profile of isolated strains:**

Only 1.42% of enterococci isolates were susceptible to all antibiotics tested in this study.

All tested isolates were susceptible to High Level Gentamycin, Ampicillin, Vancomycin and Nitrofurantoin.

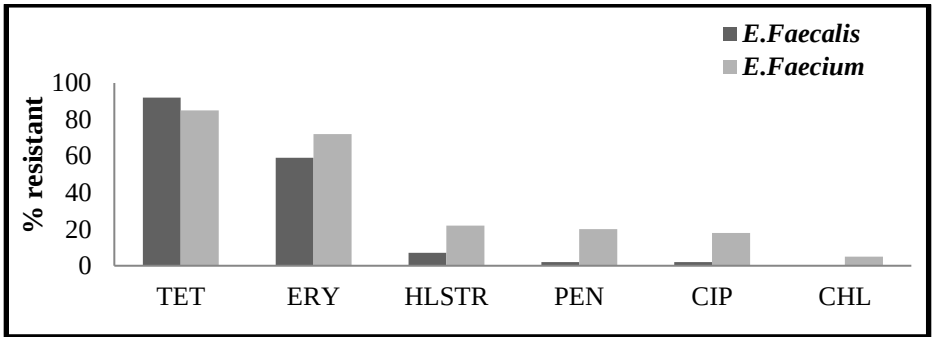
The rates of resistance to others antimicrobials were mentioned in Table 1.

Table 1

The Percentage (%) of susceptible (S), intermediate (I) and resistant (R) enterococci strains isolated from poultry.

| | S | I | R | S | I | R | S | I | R |
|---|---|---|---|---|---|---|---|---|---|
| Tetracycline | 3 | 5 | 92 | 10 | 5 | 85 | 6 | 5 | 89 |
| Erythromycin | 17 | 24 | 59 | 10 | 18 | 72 | 14 | 21 | 65 |
| HL Streptomycin | 93 | 0 | 7 | 78 | 0 | 22 | 87 | 0 | 13 |
| HL Gentamycin | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| Ampicillin | 100 | - | 0 | 100 | - | 0 | 100 | - | 0 |
| Penicillin | 98 | - | 2 | 80 | - | 20 | 90 | - | 10 |
| Vancomycin | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| Ciprofloxacin | 93 | 5 | 2 | 74 | 8 | 18 | 91 | 0 | 9 |
| Chloramphenicol | 100 | 0 | 0 | 95 | 0 | 5 | 98 | 0 | 2 |
| Nitrofurantoin | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |

Compared to *E.faecalis*, *E.faecium* isolates were significantly more resistant (P values of the following comparisons were all < 0.05) to High Level Streptomycin (7% versus 22%), Penicillin (2% versus 20%) and Ciprofloxacin (2% versus 18%) (Figure 1).




TET=Tetracycline; ERY= Erythromycin; HLSTR=high-level Streptomycin; PEN=Penicillin; CIP=Ciprofloxacin; CHL=Chloramphenicol.

Fig. 1. Comparison of drug resistance in *E.faecalis* (n=81) and *E.faecium* (n=61).

The prominent resistance phenotype in the collection was Tetracycline-Erythromycin.

Multi Drugs Resistance according to Magiorakos et al. (2012) was defined as resistance to three or more antibiotic families. Our results show that 7.4% of *E.faecalis* and 21.66% of *E.faecium* isolates were resistant to at least 3 antibiotics.

The phenotypic resistance profiles observed in *E.faecalis* and *E.faecium* are represented in table 2 and table 3 respectively.

Table 2

Phenotypic resistance profiles observed among *E. faecalis* isolates from poultry.

| | |
|---|---|
| 01 | PEN-HLSTR-ERY-TET-CIP |
| 01 | PEN-HLSTR-ERY-TET |
| 01 | HLSTR-ERY-TET-CIP |
| 03 | HLSTR-ERY-TET |
| 38 | ERY-TET |
| 04 | ERY |
| 31 | TET |

Table 3

Phenotypic resistance profiles observed among *E. faecium* isolates from poultry.

| | |
|---|---|
| 03 | PEN-HLSTR-CHL-ERY-TET-CIP |
| 07 | PEN-HLSTR-ERY-TET-CIP |
| 2 | PEN-HLSTR-ERY-TET |
| 01 | HLSTR-ERY-TET-CIP |
| 21 | ERY-TET |
| 09 | ERY |
| 17 | TET |

Color and morphology characteristics of enterococci colonies on CHROMagar Orientation allowed for their easy differentiation. Of the 141 bacterial colonies presumptively identified as enterococci on CHROMagar orientation, all of them were correctly identified with conventional methods (100%). These results are similar to those obtained by Merlino et al. (1996), Hengstler et al. (1997) and (Eun Ha Koh, M.D. and Sunjoo Kim, M.D. 2004).

Among the141 Enterococcus isolates examinated in this study, *E.faecalis* and *E.faecium*, were the only isolated species found in cecal contents from poultry. Although *E. faecalis* was isolated most frequently. This observation is consistent with some reports, where *E. faecali*s was reported the most prevalent species Aslam et al. (2012), (Yildiz and Turkyilmaz 2015), Pillay et al. (2018). However, a study conducted in Nigeria by Ngbede et al. (2017) showed that *E. faecium* was the predominant (49%) species in chicken faeces. A similar result was also observed by Ali et al. (2014) and Ünal et al. (2017), with prevalence of 66% and 33.6%, respectively.

Data on phenotypic antimicrobial resistance revealed that a high number of *E. faecalis* and *E. faecium* isolates were resistant to different classes of antimicrobials, and *E.faecium* was more resistant than *E.faecalis*, this result is similar to this obtained by Tremblay et al. (2011).

The most antibiotic resistance observed was to Tetracyclines and Macrolides. These antibiotics are frequently used for treatment and prevention in poultry, being

relatively cheap and effective against a wide variety of microorganisms Persoons et al. (2010). Thus, a high rate of resistance recorded to drugs of these antimicrobial classes. Also, Plasmids and/or conjugative transposons may carry both the tetracycline and erythromycin resistance determinants and both resistances can be maintained with any of the agents (Chopra and Roberts, 2001).

We have not isolated any enterococci resistant to high level of Gentamycin, but we have enregistered 13% of resistance to high level of Streptomycin. It was noted that 100% of Streptomycin resistant isolates also exhibited resistance to Tetracycline and Erythromycin. The resistance genes for these antibiotics could be located in the same genetic structures and the use of one of them might select for resistance to the others Aarestrup et al. (2000).

The results obtained in this study showed that all the isolated strains were sensitive to Ampicillin, and few of them had a resistance to Penicillin, although this resistance has been more frequently detected in isolates of human origin than in those of animal origin Mannu et al. (2003).

In the present study, none of the strains were resistant to vancomycin. The resistance to vancomycin is a matter of special concern, because this resistance may be transferred to more pathogenic microorganisms Pavia et al. (2000). The absence of vancomycin resistant enterococci suggests that this acquired resistance is still confined to the hospital environment. The last reported ERG in our country was in 2013 Hamidi et al. (2013).

Clinically, sensitivity to Gentamycin, Ampicillin and Vancomycin appears to be favorable, because resistance to these molecules reduces significantly therapeutic treatments in enterococcal infections Klare et al. (2003). Given that the only therapeutic choice to treat serious enterococci infections is limited to the combinations β-lactamines-Aminosides or Glycopeptides-Aminosides.

Resistance to Ciprofloxacin in *E .faecium* was higher than that enregistered in *E. Faecalis* ($P<0.001$), this result is similar to this obtained by Tremblay et al. (2011).

2% of isolates were resistant to Chloramphenicol in our study. This may be due to illegal exposes to this molecule, as it is prohibited in breeding, or to the persistence of previous resistances. All the 141 Enterococci strains were sensitive to Nitrofurantoin, as this antibiotic is not registered for the use in poultry in Algeria.

## CONCLUSIONS

The Use of CHROMagar Orientation enabled a rapid presumptive identification of enterococci, without additional tests of identification. It appears to be a medium well suited for the isolation of enterococci, and the antimicrobial resistances detected are to antibiotics widely used in farms.

**ACKNOWLEDGEMENTS.** *Authors wish to thank veterinarians of the slaughterhouses who had contributed to this work for the samples collection.*

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