ANTIBIOTIC RESISTANCE OF ENTEROCOCCI ISOLATED FROM POULTRY

Yousfi Safia, M. Bachir Pacha*
Institute of Veterinary Sciences, University of BLIDA
**Corresponding author: bachirpacha_mohamed@yahoo.fr*

Abstract: This study was conducted to evaluate the antibiotic resistance of enterococci isolated from poultry in the wilaya of Tizi Ouzou. A total of 137 enterococci isolates from poultry were tested for antibiotic susceptibility by the diffusion method. Ten antibiotics from different families were tested. High percentages of resistance to Tetracycline and Erythromycin were observed (91.97% and 73.72% respectively), Low frequencies of antimicrobial resistance to Nitrofurantoin and Penicillin with a rates of 5.83% and 7.29% respectively. Resistance was recorded for high levels of Gentamycin and Streptomycin (8.75% and 22.62% respectively). However, all strains were sensitive to Vancomycin, Ciprofloxacin, Chloramphenicol and Ampicillin.

Key words: Enterococcus, Antibiotic resistance, Nosocomial infections, Poultry.

INTRODUCTION

Antimicrobial resistance is a global issue in both human and veterinary medicine. The presence of antimicrobial resistant microorganisms in fecal material of animals is becoming a matter of great concern because these microorganisms could be transmitted to humans through a contaminated food supply Hayes et al. (2003). Among these microorganisms, enterococci that are part of the commensal flora of the gastrointestinal tract of humans and animals Kuhn et al. (2005), and are not known to be particularly pathogenic. However, their role in opportunistic and nosocomial infections has increased significantly in recent years. The two main species responsible for human infections are *Enterococcus faecalis* and *Enterococcus faecium*. Today, three elements are leading to a renewed interest in enterococci: the increasing increase in their isolation during various infections, the importance of their place in nosocomial pathology and the emergence and accumulation of antibiotic resistance mechanisms. In addition to their intrinsic resistance to many antimicrobials, including resistance to Cephalosporins, Clindamycin, and low-level to Aminoglycosides and other Beta-lactams, the enterococci have the ability to acquire other antibiotic resistance via genetic mobile elements such as plasmids, transposons, or through chromosomal exchange or mutations Hegstad et al.(2010). The latest development in resistance, which is the acquisition of glycopeptide resistance (ERG), was also the most impressive and surprising. This explains their follow-up in antimicrobial resistance surveillance programs as antibiotic resistance indicators for Gram-positive bacteria.

The purpose of our study is to evaluate the digestive carrying of different strains of enterococci in poultry slaughtered in the Wilaya of Tizi Ouzou and to determine their antimicrobial resistance profiles.

MATERIAL AND METHODS

Collection of samples: The caecal content of the birds was collected from four poultry slaughterhouses (one state and three private sector), all located in the wilaya of Tizi Ouzou, during the period from July 2017 to December 2017.

Each slaughter site was visited once during each 4-week period, in a random order. At each visit to the slaughterhouses, two flocks located in different production sites were selected whenever possible. A flock was defined as a group of birds from the same hatchery raised in a broiler house during the same period of time. Only one flock raised per production site was allowed for selection in the study. After evisceration, the caeca from the selected birds were placed in sterile plastic bags and kept on melting ice for a maximum of 8 hour prior to culture. For each flock sampled, 30 birds are randomly selected, and three pools including caecal content of 10 birds were created.

Bacterial identification: The pooled caecal sample was mixed with 25 ml of buffered peptone water until homogenization, then, a drop was collected and inoculated on a selective Bile Esculine Azide Agar (IPA). Incubation is done at 37° C in a normal atmosphere for 24 to 48 hours. After incubation, suspected enterococci colonies from each sample (small translucent colonies surrounded by a black halo) were subcultured for purity onto blood agar (Tryptic soy agar plus 5% sheep blood). Then, the identification was completed by testing for catalase, Gram-staining and by API 20 Strep system kit (Bio-Merieux, France).

Antimicrobial susceptibility testing: The antibiotic susceptibility test was determined by disc diffusion method on Mueller-Hinton agar medium (IPA), according to the recommended Clinical Laboratory Standards Institute guidelines, (CLSI, 2017). This diffusion method (Standard Antibiogram) is one of the oldest approaches to determine bacterial sensitivity to antibiotics and remains one of the most commonly used methods in routine. The antimicrobial susceptibility of enterococci was tested with a panel of 10 antimicrobial: Penicillin (10 UI), Ampicillin (10 ug), High concentration Gentamicin (120 µg), High concentration Streptomycin (300 µg), Vancomycin (30 µg), Nitrofurantoin (300 µg), Tetracycline (30 µg), Erythromycin (15 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 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). *Staphylococcus aureus ATCC® 25923 and Enterococcus faecalis ATCC® 29212* obtained from American Type Culture Collection were used as quality control organisms.

RESULTS

Identification of *Enterococcus* spp. strains: A total of 52 flocks were sampled and 156 pools including caecal from chicken broilers, turkeys and laying hen were realized. The presence of Enterococci was confirmed in 137 pools samples, representing 87.82% of specimens. The identification of strains by using the API 20 Strep system kit (Bio-Merieux, France) revealed 95 *E. faecalis*, 32 *E. faecium*, 06 *E.cecorum* and 04 *E.hirae*. The most commonly identified strain is *E.faecalis* with a rate of 69.34%, followed by *E. faecium* with a rate of 23.35%, *E.cecorum* and *E.hirae* with a rate of 04.37% and 02.92% respectively.

The results are shown in table 1.

Table 1.

Distribution of enterococci strain by type of speculation

Type of of speculation	Number of flocks	Number of pools	Isolated strains				Total
			E.faecalis	*E.faecium*	*E.cecorum*	*E.hirae*	
Broiler chicken	46	138	86	32	-	04	**122**
Turkey	04	12	06	-	04	-	**10**
Laying hen	02	06	03	-	02	-	**05**
Total	**52**	**156**	**95**	**32**	**06**	**04**	**137**

Antibiotic susceptibility profile of isolated strains: Of the 137 strains of enterococci isolated, we studied the sensitivity to the different antibiotics using the standard antibiotic susceptibility test and the results obtained are presented in Table 2. Only 5.1% of enterococci were susceptible to all tested antimicrobials. Resistance to Vancomycin, Ciprofloxacin, antimicrobials of very important in human medicine, was not found in any enterococci. All strains were susceptible to chloramphenicol and ampicillin. Most of the isolates, were resistant to Erythromycin (73.72%) and Tetracycline (91.97%). Low to intermediate frequencies of antimicrobial resistance to Penicillin (05.83%), Nitrofurantoin (07.29%), Gentamycin (high level; 08.75%), and Streptomycin (high level; 22.62%).

Table 2.

Antibiotic resistance percentages (%) of Enterococci isolated strains

	Broiler Chicken (n=122)			**Turkey (n=10)**		**Laying Hen (n=05)**		**Total (n=137)**
ATB	***E.faecalis***	***E.faecium***	***E.hirae***	***E.faecalis***	***E.cecorum***	***E.faecalis***	***E.cecorum***	
PEN	-	08	-	-	-	-	-	08 (05.83)
AMP	-	-	-	-	-	-	-	
GEN	07	04	-	-	-	01	-	12 (08.75)
STR	18	10	-	02	-	01	-	31 (22.62)
VAN	-	-	-	-	-	-	-	-
NIT	04	05	-	01	-	-	-	10 (07.29)
TET	79	29	03	06	04	03	02	126 (91.97)
ERY	68	26	01	04	01	01	-	101 (73.72)
CIP	-	-	-	-	-	-	-	-
CHL	-	-	-	-	-	-	-	-

PEN : Penicillin, AMP : Ampicillin, GEN : Gentamicin, STR : Streptomycin, VAN : Vancomycin, NIT : Nitrofurantoin, TET : Tetracycline, ERY : Erythromycin, CIP : Ciprofloxacin, CHL : Chloramphenicol.

Multidrug resistance (resistance to 03 or more antibiotics) is considered as a real threat, as 27% strains of 137 of enterococci isolates were resistant to at least three antibiotics. 14.6% of the strains were resistant to three antibiotics, 10.21% were resistant to four antibiotics and 02.19% were resistant to five antibiotics.
The results are shown in Table 3.

Table 3.

Strains of Enterococci showing multidrug resistance.

Number of resistance antibiotics	**Number of strains n=137**	**Rates of trains (%)**
03	20	14.6
04	14	10.21
05	03	02.19
Total	**37**	**27**

A total of 13 multidrug resistance profiles were obtained in our study, and reported in Table 4. *E. faecium* isolates had more multiresistant phenotypic profiles (08 different profiles) than did *E. faecalis* (05 profiles). The common multidrug resistance profile was TET ERY STR with 13 strains observed in *E.faecalis.*

Table 4.

Phenotypic profile of multidrug resistance observed in *E. faecalis* and *E. faecium*.

Bacterial Species isolates	Phenotypic multiresistant profile	Total of
E.faecalis	TET ERY GEN	02
	TET ERY STR	13
	TET ERY NIT	03
	TET ERY NIT STR	02
	TET ERY GEN STR	06
E.faecium	TET ERY STR	01
	TET ERY PEN	01
	TET PEN STR GEN	01
	TET ERY NIT STR	03
	TET ERY GEN STR	01
	TET ERY STR PEN	01
	TET ERY NIT PEN STR	02
	TET ERY NIT PEN GEN	01

DISCUSSION

E. faecalis was the predominant species (69.34% of isolates) recovered from both broiler Chicken, laying hen and turkey samples, in accordance with some reports Franz et al. (1999), Graham et al. (2009), but in contrast with others indicating *E. faecium* as the most frequent enterococci species isolated from poultry Hayes et al. (2003), Jackson et al. (2004). These differences of predominances can be attributed to geographical discrepancies or isolation methodologies Manero and Blanch, (1999), Jackson et al. (2005). Both species accounted for a large proportion (92.7%) of the enterococci isolates recovered from poultry in this study.

It should be noted, however, that the small number of isolated strains of *E. faecium* did not allow us to secondarily study the comparison of antibiotic susceptibility between the two species more closely, as *E. faecium* is generally reported to be more resistant than *E. faecalis*. Jones et al. (1995), Streff et al. (1996), SY (1996).

All strains (100%) were sensitive to Ampicillin, Ciprofloxacin and Chloramphenicol. Only a few of the isolates were resistant to Penicillin, but this mechanism of resistance has not gained wide spread importance in human medicine. The development of high-level penicillin resistance could have consequences for the treatment of enterococci infections.

We have noted in some strains a high level of resistance to aminoglycosides. This is close to the results obtained by a study conducted in Canada in 2011 by Tremblay et al. (2011). In our study, prevalence of high-level resistance to Streptomycin was much higher than high-level resistance to Gentamycin. Gentamycin is not registered for the use in poultry in Algeria; the detected prevalence of resistance was unexpected. The choice of antibiotics to treat serious enterococci infections is limited to the combinations β-lactamines-Aminosides or Glycopeptides-Aminosides, but the emergence of these strains resistant to

high levels of aminoglycoside or glycopeptide complicates the situation. In addition, the main consequence of the appearance of these high levels of resistance is the removal of the bactericidal synergistic action necessary for the treatment of severe enterococci infections. This shows the importance of systematically detecting high level resistance after isolation in the laboratory of any enterococci strain.

We have noted also high rates of resistance to Tetracycline and Erythromycin. The main reason for the high resistance to Tetracycline may be that this antimicrobial is the most commonly used in our country as both a therapeutic and non-therapeutic antimicrobials in veterinary medicine.

Few isolates were resistant to Nitrofurantoin in our study. This antimicrobial has not been reported to be used in poultry production. Therefore, Nitrofurantoin resistance cannot be clearly explained. Similar results were observed for Nitrofurantoin resistance in Portugal, which was explained by a recent massive and illicit use of furaltadone by poultry producers in this country da Costa et al. (2007).

We have not isolated any strains resistant to Vancomycin, ERG being endemic in the USA due to the use of oral Vancomycin in the treatment of Clostridium difficile infections since the 1980s. It has recently appeared in Europe following the use of Avoparcin as a growth factor in animal feed, hence the existence of the Community carry of ERG Cetinkaya et al. (2000).

In our country, the isolation of an ERG is rare. In 2007, a first case of vancomycin-resistant *E. faecalis* was reported by Aggoune et al. (2008). Also, a strain of *E.faecium* resistant to glycopeptides (ERG) was isolated from an operating wound in a patient hospitalized in a university hospital in Algiers Hamidi et al. (2013).

CONCLUSION

Multidrug resistance of *E.faecalis* and *E.faecium* isolates from poultry and the emergence of ERG and antibiotic resistance to high level of aminoglycosides are a real global threat. Vancomycin resistance was not detected, but some of the reported resistances, if spread through the food chain, would have public health implications.

Acknowledgements. *Authors wish to thank veterinarians of the slaughterhouses who had contribute to this work for the collection of samples, and for the Veterinary laboratory of D.B.K.*

REFERENCES

1. Aggoune, N., Chabani, A., Tiouit, D., Naim, M., Rahal, K. (2008). Premier cas d'*Enterococcus faecalis* résistant à la vancomycine en Algérie. Med Mal Infect, 38 : 557-8.
2. Cetinkaya, Y., Falk, P., Mayhall, C.G. (2000). Vancomycin-resistant Enterococci. Clin Microbiol Rev, 13: 686-707.
3. Clinical and laboratory standards institute, (CLSI, 2017), Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA.
4. da Costa, P. M., M. Oliveira, A. Bica, P. Vaz-Pires, and F. Bernardo. 2007. Antimicrobial resistance in Enterococcus spp. and Escherichia coli isolated from poultry feed and feed ingredients. Vet. Microbiol. 120:122–131.
5. Franz, C. M., W. H. Holzapfel, and M. E. Stiles. 1999. Enterococci at the crossroads of food safety? Int. J. Food Microbiol. 47:1–24.

6. Graham, J. P., S. L. Evans, L. B. Price, and E. K. Silbergeld. 2009. Fate of antimicrobial-resistant enterococci and staphylococci and resistance determinants in stored poultry litter. Environ. Res. 109:682–689.
7. Hamidi, M., Ammari, H., Ghaffor, M., Benamrouche, N., Tali-Maamar, H., Tala-Khir, F., Younsi, M., Rahal, K. (2013). Émergence d'Enterococcus faecium résistant aux glycopeptides en Algérie: à propos d'un cas. Ann Biol Clin, 71(1) : 104-6.
8. Hayes, J. R., L. L. English, P. J. Carter, T. Proescholdt, K. Y. Lee, D. D. Wagner, and D. G. White. (2003). Prevalence and antimicrobial resistance of Enterococcus species isolated from retail meats. Appl. Environ. Microbiol. 69:7153–7160.
9. Hegstad, K., Mikalsen, T., Coque, T.M., Werner, G., Sundsfjord, A. (2010). Mobile genetic elements and their contribution to the emergence of antimicrobial resistant Enterococcus faecalis and Enterococcus faecium. Clinical Microbiology and Infection, 16: 541–554.
10. Jackson, C. R., P. J. Fedorka-Cray, J. B. Barrett, and S. R. Ladely. (2004). Genetic relatedness of high-level aminoglycoside-resistant enterococci isolated from poultry carcasses. Avian Dis. 48:100–107.
11. Jackson, C.R., Fedorka-Cray, P.J., Jackson-Hall, M.C., Hiott, L.M., (2005). Effect of media, temperature and culture conditions on the species population and antibiotic resistance of enterococci from broiler chickens. Letters in Applied Microbiology 41,262–268.
12. Jones, R.N., Sader, H.S., Erwin, M.E., Anderson, S.C. (1995). Emerging multiply resistant enterococci among clinical isolates: Prevalence data from 97 medical center surveillance study in the United States. Diagn. Microbiol. Infect. Dis., 21 (2): 85-93.
13. Kuhn, I., Iversen, A., Finn, M., Greko, C., Burman, L.G., Blanch, A.R., Vilanova, X., Manero, A., Taylor, H., Caplin, J., Domínguez, L., Herrero, I.A., Moreno, M.A., Möllby, R. (2005). Occurrence and relatedness of vancomycin-resistant enterococci in animals, humans, and the environment in different European regions. Appl Environ Microbiol, 71(9): 5383-90.
14. Manero, A., Blanch, A.R., (1999). Identification of Enterococcus spp. With a biochemical key. Applied and Environmental Microbiology, 65, 4425–4430.
15. Streff, K., Jean Pierre, H., Darbas, H., Paillisson, J. (1996). Enterocoques au CHRU de Montpellier durant le mois de septembre 1993 : espèces isolées, répartition en fonction du prélèvement, rôle pathogène, sensibilité aux bêtalactamines, aminosides, glycopeptides. Méd. Mal. Infect, 26 : 704-13.
16. Sy, K.R. (1996). Souches bactériennes et résistance aux antibiotiques : données actuelles au CHU A. Le DANTEC de Dakar Thèse, pharmacie, Dakar, N°61.
17. Tremblay, C.L, Letellier, A., Quessy, S., Boulianne, M., Daignault, D., Archambault, M. (2011) Multiple-Antibiotic Resistance of Enterococcus faecalis and Enterococcus faecium from Cecal Contents in Broiler Chicken and Turkey Flocks Slaughtered in Canada and Plasmid Colocalization of tetO and ermB Genes. Journal of Food Protection, 74(10): 1639-48.