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Multiple Antibiotic Resistance (MAR) Index of Enterococcal Isolates Isolated from Healthy Poultry Faeces in Bikaner Region

Kanchan Jangir, B.N. Shringi, Mukesh Beriwal, Ramhari Meena, Sunil Maherchandani and S.K. Kashyap

Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Science, Bikaner-334001, India.

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The study was carried out with an objective to establish a baseline for antimicrobial susceptibility of *Enterococcus* isolates recovered from healthy poultry faeces in Bikaner region. The isolates were subjected to antibiotic sensitivity test by Kirby Bauer disk diffusion method using 19 different antibiotics belonging to different classes. Based on the resistance pattern of the isolates, the Multiple Antibiotic Resistance (MAR) Index was calculated. The results of the study revealed that almost all of the isolates exhibited Multi Drug Resistance (MDR) character and all the isolates had a very high MAR Index, suggesting the origin of the isolates to be of high antibiotic usage.

Key words: *Enterococcus*, MAR index, MDR isolates.

Uncontrolled usage of antimicrobials in food animals as poultry has been recognized as the most important factor that determines the development and spread of resistant microorganisms**.** Poultry have increasingly been associated with carriage of multi-drug resistant enterococci. More than 26% of the antibiotics used in veterinary practice were intended for poultry, mainly for broilers, resulting in a yearly exposure of 430 mg of antibiotic/kg/yr of poultry (Van den Bogaard *et al.*, 2000). This was considerably higher than the antibiotic usuage in other food animal populations (Van den Bogaard *et al.*, 2000). Hence, antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria (Van den Bogaard *et al.*, 2002*).* The results of previous studies have indicated that the use of avoparcin, gentamicin (GEN), and virginiamycin for growth promotion and therapy in food animals has led to the emergence of vancomycin- and gentamicin-resistant enterococci (VRE and GRE) and Quinupristin-Dalfopristin (QD) -resistant *E. faecium* in animals and meat (Hammerum *et al.*, 2010). The animal intestinal tract can act as a reservoir for vancomycin resistant *Enterococcus* (VRE) and food-producing animals can directly transfer VRE to humans via the food chain (Kuhn *et al.*, 2005). Animals, especially livestock, such as chickens and pigs, have consistently been reported as the reservoirs for VRE (Vignaroli *et al.*, 2011). Several studies suggest that poultry products could be a source of vancomycin resistant *Enterococcus* (VRE) in humans through the food chain (Roberdo *et al.*, 2000). Multidrug resistance is common among enterococci and presents a formidable treatment problem (Donabedian *et al.*, 2003).

* To whom all correspondence should be addressed.
Tel. : 09785607106
E-mail: kanchanjangid87@gmail.com

Multiple antibiotic resistance (MAR) indexing has been shown to be a cost effective and valid method of bacteria source tracking. MAR analysis has been used to differentiate bacteria from different sources using antibiotics that are commonly used for human therapy. Compared to other methods of bacteria source tracking such as genotypic characterization, the MAR indexing method is cost effective, rapid and easy to perform. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used.

MATERIALS AND METHODS

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of pre-identified enterococcal isolates was performed using the disc diffusion method (Bauer *et al.*, 1966). Values obtained were interpreted according to the Clinical and Laboratory standards Institute (CLSI, 2010) into resistant, intermediate and sensitive categories.

MAR (Multiple Antibiotic Resistance) Index Study

The MAR Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected (Jayaraman Sathishkumar *et al.*, 2012).

Identification of MDR (Multi Drug Resistance)

Multi Drug Resistance is defined as resistance to least two classes of antibiotics among all the tested antibiotics. The Multi Drug Resistance (MDR) characters of the isolates were identified by observing the resistance pattern of the isolates to the antibiotics.

RESULTS

Antimicrobial profile of *Enterococcus* isolates as obtained from Kirby Bauer disk diffusion method was interpreted as per CLSI standards is shown in table-I. Absolute resistance was obtained for oxacillin, clindamycin and cefepime (IV generation cephalosporin). A total of 95% isolates were resistant to tetracycline, 90% towards colistin and 85% towards nalidixic acid. The most susceptible antibiotics as per this study were ampicillin, Piperacillin/tazobactum and penicillin-G. The MAR indexes of the isolates were calculated and noted (Table - II & III).

DISCUSSION

Resistance in commensal bacteria often is high to broad spectrum of antimicrobials (Goldstein *et al.*, 2001). Those bacteria are left uncontrolled, as they do not cause any clinical signs of diseases. That helps them to survive in different conditions by pressure of different quantities and spectrum of antimicrobials. Hence their level of resistance is considered to be a good indicator for selection pressure by antibiotic use and for resistance problems to be expected in pathogens. Monitoring the prevalence of resistance in indicator bacteria such as faecal *Enterococcus* and *Escherichia coli* in different populations, makes it feasible to compare the prevalence of resistance and to detect transfer of resistant bacteria or resistance genes from animals to humans and vice versa (Lukaova *et al.*, 2003). Enterococci usually are found in large numbers in food of animal origin, such as cattle, pig, and poultry carasses (Hammerum *et al.*, 2010) and their presence is an indication of faecal contamination, which commonly occurs during slaughter of the animals (Hammerum *et al.*, 2010). Due to their resistance to freezing, low pH, and moderate heat treatment, the *Enterococci* have been suggested as an indicator in some types of food products (Banwart *et al.*, 1989) and also associated with processed & heat treated food materials (Foulquie-Moreno *et al.*, 2006). Heating of processed meat may confer a selective advantage on enterococci, as they are among the most tolerant of non-sporulating bacteria (Bradley and Fraise, 1996).

All the enterococcal isolates in this study showed resistance to multiple antibiotics and MAR value calculated was much higher than 0.2 *i.e.* ranged from 0.31 to 0.84 which indicated a high risk of antibiotic contamination. MAR index obtained indicated that multi-drug resistance was predominant among all isolates. Similar data was verified by Son *et al.* (1999), who reported MAR rates ranging from 0.2 to 0.9 in *Enterococcus* of beef origin and by Carvalho *et al.*

Table 1. Susceptibility of the Isolates to antibiotics as per CLSI standards:

S.No.	Antibiotic disc	Concentration (mcg or unit/disc)	*Enterococcus* isolates Resistant	Intermediate	Sensitive
1.	Ampicillin	10	17.5%(7)	-	82.5%(33)
2.	Clindamycin	2	100%	-	-
3.	Cefepime	30	100%	-	-
4.	Cefazolin*	30	65%(26)	-	35%(14)
5.	Cefotaxime	30	65%(26)	12.5%(5)	22.5%(9)
6.	Cephalothin	30	40%(16)	12.5%(5)	47.5%(19)
7.	Chloramphenicol	30	27.5%(11)	10%(4)	62.5%(25)
8.	Ciprofloxacin	5	37.5%(15)	37.5%(15)	25%(10)
9.	Colistin	10	90%(36)	-	10%(4)
10.	Cotrimoxazole	25	82.5%(33)	-	17.5%(7)
11.	Gemifloxacin	5	57.5%(23)	17.5%(7)	25%(10)
12.	Levofloxacin	5	7.5%(3)	25%(10)	67.5%(27)
13.	Nalidixic acid	30	85%(34)	2.5%(1)	12.5%(5)
14.	Oxacillin	1	100%	-	-
15.	Penicillin-G	10	27.5%(11)	-	72.5%(29)
16.	Piperacillin/Tazobactum*	100/10	10%(4)	12.5%(5)	77.5%(31)
17.	Rifampicin	5	62.5%(25)	22.5%(9)	15%(6)
18.	Tetracycline	30	95%(38)	2.5%(1)	2.5%(1)
19.	Trimethoprim	5	47.5%(19)	40%(16)	12.5%(5)

*- No CLSI standards; interpretation was done on the basis of literature. (Cefazolin-Actor *et al*., 1974 and Piperacillin/Tazobactum-Abdulla *et al*., 2006)

Table 2. MAR index of enterococcal isolates

S.No.	Isolate. No.	No.of antibiotics to which isolate is resistant(a)	MAR index=a/b	S.No.	Isolate. No.	No. of antibiotics to which isolate is resistant(a)	MAR index=a/b
1.	CK1	11	0.578	21	CK24	7	0.368
2.	CK2	12	0.631	22.	CK25	12	0.631
3.	CK3	9	0.473	23.	CK26	8	0.421
4.	CK4	8	0.421	24.	CK27	6	0.315
5.	CK6	13	0.684	25.	CK28	8	0.421
6.	CK7	12	0.631	26.	CK29	16	0.842
7.	CK8	9	0.473	27.	CK30	12	0.631
8.	CK9	11	0.578	28.	CK31	11	0.578
9.	CK10	12	0.631	29	CK32	11	0.578
10.	CK11	12	0.631	30.	CK33	8	0.421
11.	CK12	14	0.736	31.	CK34	10	0.526
12.	CK13	12	0.631	32.	CK35	13	0.684
13.	CK14	12	0.631	33.	CK36	12	0.631
14.	CK15	13	0.684	34.	CK37	13	0.684
15.	CK16	14	0.368	35.	CK38	11	0.578
16.	CK17	9	0.473	36.	CK39	7	0.368
17.	CK19	9	0.473	37.	CK40	7	0.368
18.	CK21	10	0.526	38.	CK43	8	0.421
19.	CK22	13	0.684	39.	CK45	13	0.684
20.	CK23	14	0.736	40.	CK46	12	0.631

NOTE: Total number of antibiotics to which isolates were subjected = 19 (b)

Table 2. MAR index values with No. of isolates

S.No.	MAR index	No. of isolates
1.	0.315	1
2.	0.368	4
3.	0.421	5
4.	0.473	3
5.	0.526	2
6.	0.578	5
7.	0.631	10
8.	0.684	6
9.	0.736	3
10.	0.842	1

(2014) who reported MAR levels in *Enterococcus* from coastal water ranged from 0.25 to 0.87. All enterococcal isolates of cattle faeces origin showed an MAR index above 0.2 (water; 0.58 to 0.68 and faeces; 0.6 to 1) in a study conducted by Ramatlhape *et al*. (2006). This high range of MAR index among almost all isolates can be explained by the fact that poultry is the most commonly used food animal and use of antibiotics as growth promoters in these food animals lead to the emergence of resistance to multiple drugs. Multiple antibiotic resistance (MAR) in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype (Daini *et al.,* 2005).

All these findings have implications for the choice of antibiotics for empiric management of infections and continuous supervision of antibiotic susceptibility patterns. It was speculated in this study that there could be a development of potential reservoirs of antibiotic resistance in poultry farms. In order to prevent the distribution of MAR organisms or their transferable resistance genes, a judicious use of antibiotics is necessary in veterinary medicine, animal husbandry and human medicine. High prevalence of multidrug resistance indicates a serious need for broad-based, local antimicrobial resistance surveillance and planning of effective interventions to reduce multidrug resistance in such pathogens. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless inappropriate uses of drugs are curtailed and continuous education of infection control practices maintained.

REFERENCES

1. Abdulla, F.E., Abdulla, E.M. Antibiotic options for *Enterococcus faecalis* infections. *Pak J Med Sci*. 2006; **22 (3):** 286-290.
2. Actor, P., Guarini, J., Uri, J., Dickson, J., Pauls, J.F., Weisbach, J.A. Disk Susceptibility Studies with Cefazolin and Cephalothin. *Antimicrobial agents and chemothemrapy.* 1974; **5(1):** 63-67.
3. Banwart, G.J. *Basic food microbiology.* 1989; **383:** 371-392.
4. Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol*. 1966; **45(4):** 493-496.
5. Bradley, C.R., Fraise, A.P. Heat and chemical resistance of enterococci. *Journal of Hospital infections.* 1996; **34**: 191-196.
6. Carvalho E.M.R., Costa R.A., Araujo A.J.G., Carvalho, F.C.T., Pereira, S.P., Sousa, O.V., Vieira, R.H.S.F. Multiple-antibiotic resistance of Enterococcus isolated from coastal water near an outfall in Brazil. *African journal of microbiology research*. 2014; **8(17)**: 1825-1831.
7. Daini, O.A., Ogbolu, O.D., Ogunledun, A. Quinolone resistance and R-plasmids of some Gram negative enteric bacilli. *Afr J Clin Exper Miccrobiol.* 2005; **6(1):** 14-20.
8. Donabedian, S.M., Thal, L.A., Hershberger, E., Perri, M.B., Chow, J.W., Bartlett, P., Jones ,R., Joyce, K., Rossiter, S., Gay, K., Johnson, J., Mackinson, C., Debess, E., Madden, J., Angulo, F., Zervos, M.J. Molecular characterization of gentamicin-resistant enterococci in the United States: evidence of spread from animals to humans through food. *J. Clin. Microbiol*. 2003; **41**: 1109–1113.
9. Foulquie-Moreno, M.R., Sarantinopoulos, P., Tsakalidou, E., De Vuyst, L. The role and application of enterococci in food and health. *International Journal of Food Microbiology.* 2006; **106**: 1-24.
10. Goldstein, C., Lee, M.D., Sanchez, S., Hudson, C., Phillips, B., Register, B., Grady, M.M., Liebert, C., Summers, A.O., White, D.G., Maurer J.J. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals and exotics. *Antimicrob. Agents Chemother*. 2001; **45:** 723-726.

11. Hammerum, A.M., Lester, C.H., Heuer, O.E. (2010). Antimicrobial-resistant enterococci in animals and meat: a human health hazard? *Foodborne Pathog. Dis.* **7**: 1137–1146.
12. Jayaraman, S.K., , Manoharan, M., Ilanchezian, S., Sekher, R., Sathyamurthy. Plasmid analysis and prevalence of Multidrug resistant Staphylococcus aureus reservoirs in Chennai city, India. *Asian Journal of Pharmacy and Life Science.* 2012; **2** (2): 2231 – 4423.
13. Kuhn, I., Iversen, A., Finn, M.C., Greko, L.G., Burman, A.R., Blanch, X., Vilanova, A., Manero, H., Taylor, J., Caplin, L., Domínguez, I.A., Herrero, M.A., Moreno Mollby, R. Occurrence and relatedness of vancomycin-resistant enterococci in animals. *Environ. Microbiol*. 2005; **71:** 5383-5390.
14. Lukaova, J., Ustaakova, A. Review Article Enterococci and Antibiotic Resistance. *Acta vet. Brno* 2003; **72**: 315–323.
15. Ramatlhape, L.L.N. The assessment of multiple antibiotic resistant enterococci in communal and commercial cattle faecal samples and their water sources in Mafikeng, North-West Province, RSA. *B. Sc. Hons. Thesis.* 2006.
16. Robredo, B., Singh, K.V., Torres, C., Murray, B.E. Streptogramin resistance and shared pulsed-field gel electrophoresis patterns in Van A-containing *Enterococcus faecium* and *Enterococcus hirae* isolated from humans and animals in Spain. *Microb. Drug Resist*. 2000; **6**: 305-311.
17. Son, R., Nimita, F., Rusul, G., Nasreldin, E., Samuel, L., Nishibuchi, M. Isolation and molecular characterization of vancomycin resistant *Enterococcus faecium* in Malaysia. *Lett. Appl. Microbiol*. 1999; **29:** 118–122.
18. Van den Bogaard, A.E. Veterinary use of antibiotics in the Neteherlands-facts and figures. *Tijdschrift Diergeneeskund*. 2000; **125**: 527-30.
19. Van den Bogaard, A.E., Willems, R., London, N., Top, J., Stobberingh, EE. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. *J. Antimicrob. Chemother*. 2002; **49:** 497–505.
20. Vignaroli, C., Zandri, G., Aquilanti, L., Pasquaroli, S., Biavasco, F. Multidrug-resistant enterococci in animal meat and faeces and co-transfer of resistance from an *Enterococcus durans* to a human *Enterococcus faecium*. *Curr. Microbiol*. 2011; **62:** 1438–1447.