Antibiogram profile based dendrogram analysis of Escherichia coli serotypes isolated from bovine mastitis

Sumathi B.R, Amitha R. Gomes and G. Krishnappa

Department of Veterinary Microbiology,
Karnataka Veterinary, Animal and Fisheries Sciences University,
Hebbal, Bangalore - 560024.

Environmental bovine mastitis caused by coliform bacteria has increased in many herds and countries. *Escherichia coli* constitute the majority of these coliform bacteria. *E. coli* originate from the cow's environment and infect the udder via the teat canal (Eberhart, 1979). Many authors have reported *E. coli* as the second most common etiological agent causing mastitis in cows following *Staphylococcus aureus* (Bansal *et al*., 1990 and Char *et al*., 1993). Higher incidence of *E. coli* mastitis may be due to poor hygienic conditions or intensive use of antimicrobials targeted against Gram positives for mastitis control (Radostits *et al.,* 2000). As a result coliform mastitis remains as one of the most difficult diseases to treat in the modern dairy industry. Curative therapy with antibiotics remains only moderately effective and depends on the stage at which the disease is treated. The most important factor in the control of *E. coli* mastitis is the emergence of multiple drug resistant strains. Indiscriminate use of antibiotics in the treatment of mastitis has lead to the emergence of drug resistant strains. Furthermore, the transmissibility of antimicrobial resistance and virulence factors by conjugation may contribute to the development and dissemination of pathogenic *E. coli* strains (Holmberg *et al*., 1984). Therefore, it is necessary to select suitable antibiotics, preferably after antibiotic sensitivity testing and using such antibiotics at an adequate dose for sufficient duration to ensure effective treatment and control of *E. coli* mastitis. The aim of this study was to determine the *in vitro* antimicrobial susceptibility of *E. coli* bacteria isolated from clinical bovine mastitis in Bangalore and their dendrogram based analysis for phylogenetic analysis.

Material and Methods

Sources of milk samples: Sixty milk samples from clinical mastitis cases were collected from different dairy farms located in and around Bangalore and from cases that were presented in the clinics, Veterinary College, Hebbal, Bangalore. Milk samples were aseptically collected from the affected quarters of the cows before any antimicrobial treatment and cultured using standard methods.

Cultural Examination and Identification of Isolates: For Bacteriological examination, approximately 0.01 ml of milk sample was inoculated on to blood agar, nutrient agar, MacConkey's agar and Eosine Methylene Blue agar plates and the plates were incubated at 37°C for 24 to 48 hours. The bacteria isolated were identified and *E. coli* isolates were confirmed on the basis of their cultural, morphological and biochemical characteristics as described by Cruickshank *et al.* (1975).

Serotyping: Pure cultures of *E. coli* isolates were grown on nutrient agar slants and sent to National Salmonella and Escherichia center, Central Research institute, Kasauli, Himachal Pradesh, India for serotyping. Thereafter, cultures were routinely subcultured into nutrient agar and maintained as per standard procedures.

Antimicrobial susceptibility testing: Minimal inhibition concentration (MIC) values of the serotypes were analyzed for fourteen different antimicrobials (M/s Hi Media Laboratories Ltd., Mumbai) namely ampicillin (30µg), chloramphenicol (30 µg), cephalexin (30 µg), ciprofloxacin (10µg), cloxacillin (30µg), colistin (Methane sulphonate (25 µg), enrofloxacin (10µg), nitrofurantoin (300µg), furazolidone(50µg), gentamicin (30µg), tetracycline (30µg), neomycin (30µg), streptomycin (10 µg) and sulphadiazine (300 µg).

The disc diffusion method as described by Miles and Amyes (1996) was employed and the interpretation was made as per the zone size interpretation chart provided by the manufacturer of discs. The sensitivity pattern was scored as resistant

(0), less sensitive (1), moderately sensitive (2), and sensitive (3).

Statistical Analysis: The scored antibiogram data were analysed with a computer package STATISTICA. The dissimilarity matrix was developed using Squared Euclidean Distance (SED) that estimated all pair wise differences in the sensitivity pattern (Sokal and Sneath, 1973) and the dendrogram was computed based on Ward's method of clustering.

Results and Discussion

Prevalence of E. coli serotypes**:** Among the isolates typed, fourteen were typed as 'O' serogroups and one was untypable. Among the 'O' serogroups 9 different 'O' serotypes have been identified namely O18 (5), O21 (2) and O20, O171, O109, O11, O172, O128, O29 (one each). The most predominant serotype was O18, accounting for 33.33 %.

High prevalence of *E. coli* in mastitis milk samples has also been reported by Deborah *et al.* (1991) and Balakrishnan *et al*. (2004). However they have not reported the serogrouping of these serotypes.

E. coli serotype O18, reported in this study as most prevalent one has also been reported by Zhao and Yang (1999), Pandey *et al.* (1998) and Gao *et al.* (1999) as a pathogenic serotype in poultry species associated with colibacillosis.

Antibiogram profile

The dendrogram based on antimicrobial drug sensitivity pattern of nine *E. coli* serotypes is represented in Fig 1 and the accessions are clearly divided into two major clusters, A and B. The maximum dissimilarity distance between the two major clusters A and B was 29 units.

The cluster A can be grouped into 2 subclusters A1 and A2 at a linkage distance of 23 units. The subcluster A1 is further divided into two subgroups A1.1 and A1.2 at a linkage distance of 6 units. The subgroup A1.1 consists of two serotypes O21 and O171 which were resistant to ampicillin, sulphadiazine and furazolidone and subgroup A1.2 consists of two serotypes O11 and O29 which were resistant to antibiotic cloxacillin , nitrofurantoin and sensitive to furazolidone when compared to subgroup A1.1 (Table 1).

The subcluster A2 is further divided into subgroups A2.1 and A2.2 at a linkage distance of 14 units. The subgroup A2.1 consisted of two serotypes O109 and O20 which were resistant to ampicillin, sulphadiazine, tetracycline and furazolidone. The subgroup A2.2 consisted of two serotypes O172 and O128 which were resistant to cephalexin when compared to subgroup A2.1 (Table-1).

The cluster B consisted of only one isolate O18 which differed from cluster A by having resistance to all the antibiotics except chloramphenicol, ciprofloxacin and enrofloxacin.

The study of relatedness among various serotypes of *E. coli* is an important area in the management of infectious diseases in more than one way. This can be achieved through phylogenetic analysis based on dendrogram approach. This helps to understand the evolution of newer antibiotic resistant types and its importance in the epidemiological investigations in identifying the source of disease.

In the present study, it was found that the O18 serotype differed from serotypes of other major clusters by having resistance to all the antibiotics except for chloramphenicol, enrofloxacin and ciprofloxacin, and O18 has been found to be predominant serotype among *E. coli* isolates from clinical bovine mastitis, constituting for 33.33% of *E. coli* isolates. Studies need to be undertaken on development of mastitis vaccine, where in serotype O18 could be a potential vaccine candidate in view of its high prevalence.

From the above dendrogram analysis it can be concluded that the *E. coli* O18 serotype is the parent strain (species) of the genus as this serotype came in the root cluster B with its unique identity. All other eight serotypes constituting cluster A probably might have originated from parental O18 serotype in the process of genetic evolution. Thus dendrogram analysis revealed the possibility of this method as a taxonomic tool.

Most of the serotypes in the present study were found to be resistant to ampicillin, cloxacillin, colistin, neomycin and furazolidone. Resistance pattern similar to our study was also reported by Mini *et al.* (2005) and Lalrintluanga *et al.* (2003). Development of multiple drug resistance among most of these serotypes may be related to transmission of R factor (McKay *et al*., 1965) which is extrachromosomal genetic determinants 'plasmids'. *E. coli* serotypes often contain multiple plasmids that may contain any number of antibiotic resistant genes (Wooley *et al*., 1992).

From the above antibiotic resistance pattern it can be concluded that indiscriminate and frequent use of these antibiotics in animals could be the

reason for their ineffectiveness against *E. coli.* Hence, this study emphasizes judicious selection of antibiotics, preferably after antibiotic sensitivity testing and using such antibiotics at an adequate dose for sufficient duration to ensure effective treatment and control of mastitis caused by *E. coli.* In conclusion, the dendrogram provided an insight into the relatedness of different serotypes of *E. coli* based on antibiogram profile. This approach is perhaps the most realistic and presents a quick bird's eye-view of the complex inter-relatedness among *E. coli* serotypes with respect to their sensitivity and resistance to various antibiotics.

References

1. Balakrishnan, G., Madhavan Unny, Dorairajan, N. and Subramaniah M., 2004, *Indian Vet. J.,* **81**:1166-1167.
2. Bansal, B.K., Singh,K.B., Jand,S.K., Nauriyal,D.c. and Randhawa, S.S 1990, *Indian J. Dairy Sci*., **43**:355.
3. Char, N.L., Rao, M.R.K. and Rajeswari, K.R. 1993, I*ndian Vet. J.,* **70**:378-379.
4. Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. 1975, Medical Microbiology. Vol. II, **12th edn.** CrurchillLivingstone, New York, pp. 31-57 and 96-218.
5. Deborah, A.T., Larry smith, K., Hogan, J.S. and Pamela S. Schoenberger., 1991, *Am. J. Vet. Res.,* 52:184-188.
6. Eberhart, R.J.1979, Antimicrobial use in bovine mastitis. Pages 712-734 in Antimicrobial Therapy in Veterinary Medicine. **3rd ed.** J. F. Prescott, J.D. Baggot, and R. D. Walker, eds. Iowa State Univ. Press, Ames.
7. Gao, S., Liu, X. F., Zhang, R.K., Teng, F., Lu, Y. H., 1999, *Acta. Microbiologica. Sinica.*, **39** :226-233.
8. Holmberg, S.D., Osterman, M.T. and Senger, K.A., 1984, N.Engl.J.Med., 311:617-622.
9. Lalrintluanga, C., Esther, L.R. and Hamarkunga., 2003, *Indian Vet. J.,* **80**: 931-932.
10. Mckay, K.A., Rubrike, H.I. and Barnum, D.A., 1965, *Can. Vet. J.,* **16**:103-111.
11. Miles and Amyes., 1996, Laboratory control of antimicrobial therapy: (Cited in) *Practical Medical Microbiology*, pp. 151-178.
12. Mini, M., Venkatesan, R.A and Purushothaman, V., 2005, *Indian Vet. J.,* 82:587-590.
13. Pandey, G.S., Tuchili, L.M., Kaneuchi, C., Ulaya, W. and Nyeleti, G., 1998, *Indian Vet. J.,* **75**: 754-755.
14. Radostits, O.M., Gay, G.C., Blood, D.C and Hinchkiliff, K.W., 2000, *Veterinary Medicine.* 9thedn. ElBS and Bailliere Tindall. *pp*. 563-618.
15. Sokal, R. R. and Sneath, P. H. A., 1973, Principles of numerical taxanomy, W. H. Freeman and Co., San Francisco, USA.
16. Wooley, R.E., Spears, K.R., Brown, J., Nalan, L.K. and Dekich, M.A., 1992, *Avian Dis.,* **36**:348-352.
17. Zhao, X.R. and Yang, H.C., 1999, *Chinese J. Vet. Med.,* **25**:3-5.

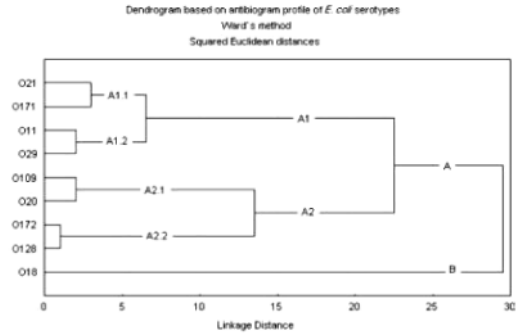

Table 1. Antibiotic sensitivity pattern of different E.coli serotypes isolated from clinical bovine mastitis.

Antibiotics	Serotypes of E.coli								
	O21	O171	O109	O11	O172	O128	O29	O20	O18
Ciprofloxacin	3	3	3	3	3	3	3	3	3
Chloramphenicol	3	3	3	3	3	3	3	3	2
Enrofloxacin	2	3	2	2	3	3	3	2	3
Gentamicin	2	3	2	2	3	3	2	2	0
Cephalexin	2	2	2	3	0	0	0	2	0
Streptomycin	1	1	1	1	2	2	1	0	0
Neomycin	1	1	1	1	2	2	1	1	0
Nitrofurantoin	1	1	2	0	1	1	0	2	0
Colistin	1	1	2	1	1	1	0	2	0
Tetracycline	1	1	0	1	0	0	1	0	0
Cloxacillin	1	1	0	0	0	0	0	0	0
Furazolidone	0	0	0	1	0	0	1	0	0
Ampicillin	0	0	0	0	0	0	0	0	0
Sulphadiazine	0	0	0	0	0	0	0	0	0

0= Resistant 1= Low sensitive 2= Moderately sensitive 3= Highly sensitive