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ANTIMICROBIAL ACTIVITY OF PROTEUS BACTERIA ISOLATED FROM RAW MILK OF PAONTA VALLEY

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ABSTRACT

This study was carried out from 8th Mar 2007 to 8th Dec 2009. A total of 65 consecutive *Proteus* recovered during the study period in 360 milk samples of different area of Paonta valley, 40 isolates were ESBL producer and 25 isolates were non-ESBL producers. Detection of extended spectrum β-lactamase producing *Proteus* in raw milk and milks products was carried out by Kirby Bauer disc diffusion method, on Muller Hinton Agar. A susceptibility disk containing Piperacillin\ Tazobactum was placed as the inhibitor of β-lactamase in the center of the plate, Piperacillin were placed 30mm from the Piperacillin\ Tazobactum disk. Enhancement of zone of inhibition of disc of Piperacillin alone towards the disc containing Piperacillin\ Tazobactum, showing a figure of eight impression were considered as ESBL producer. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as control strains.
The prevalence of extended spectrum β-lactamase producing *Proteus* in raw milk and milks products of Paonta valley was 11%.

KEYWORDS

ESBL, Antibiotic, *Enterobacteriaceae*.

INTRODUCTION

β-lactam antibiotics are among the most frequently prescribed antimicrobial agents world wide. The emergence of resistance to these agents in the past two decades has resulted in major clinical crisis (1-2).Gram negative bacteria resistant to agent such as extended-spectrum Cephalosporins, Monobactam, Carbapenems and β-lactam - β-lactamase inhibitor combinations have emerged through the production of a variety of β-lactamase, alternation in the Penicillin-binding proteins, outer membrane permeability and combinations of multiple mechanisms of resistance. This increase has paralleled the introduction, administration and overuse of β-lactam uses (3). Resistance to β-lactam antimicrobial agents, especially extended spectrum Cephalosporins and other antimicrobial agent among clinical isolates of Gram negative bacteria is on the rise worldwide (4,5). These antimicrobial resistant pathogens include extended–spectrum Cephalosporin resistant *Escherichia coli, Klebsiella pneumoniae, Proteus, Enterobacter cloacae, Serratia marcescens* and *Citrobacter freundii, Pseudomonas aeruginosa*(6). *Proteus mirabilis* isolates are commonly susceptible to most antibiotics,

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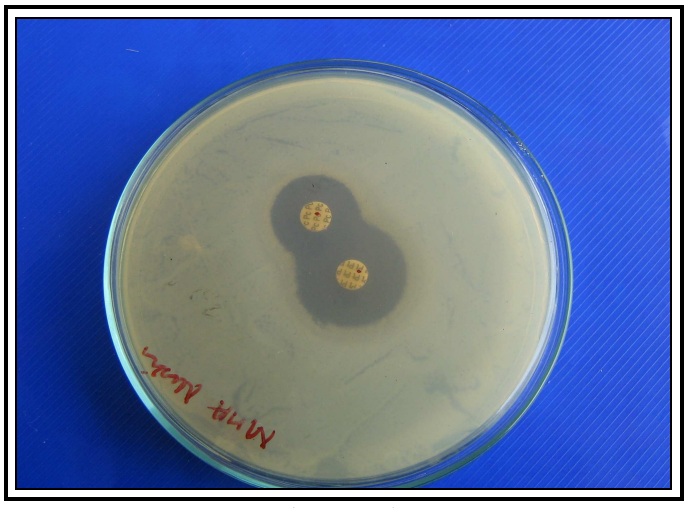
including β-lactam. However, acquired resistance to the above mentioned drugs has increased over the last few years, due to the production of extended- spectrum β-lactamase (ESBL).A 3-year survey conducted in France from 1988 to1990 showed that only 0.8% of *P. mirabilis* strains produced ESBL (14).Subsequently the prevalence of ESBL positive strains has increased to 6.9% in France and 9.5% in the U.S.[15, 16] A national wide survey showed that, among ESBL producing *Enterobacteriaceae*, *P. mirabilis* was the second most frequent species in Italy(17). The prevalence of ESBL positive strains of *P. mirabilis* is 8.8% in Italy(1).The objective of this study was to determine the prevalence of extended spectrum β-lactamase producing *Proteus* in raw milk and milks products of Paonta valley.

MATERIALS AND METHODS

A total of 40 ESBL-producing *Proteus sp*. were recovered from 360 samples of raw milk of Paonta valley during the study period from 8th March 2007 to 8th Dec 2009. The samples were received initially inoculated on MacConkey agar, it gives non–lactose fermenting colourless colonies, and on HEA, it gives green colony with or without black centre. The isolates were identified on the basis of colony morphology and biochemical reactions. ESBL production was detected by **Double Disc Diffusion** Method by placing a Piperacillin disc alone towards the disc containing Piperacillin/ Tazobactum, showing a figure of 8 impressions were considered as ESBL producers. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as control strains.

RESULTS

A total of 40 isolates were ESBL producers in 360 samples of raw milk of Doon valley. So the prevalence of *Proteus sp*. in raw milk collected from different location of Paonta valley is 11%. Enhancement of zone of inhibition of discs of Piperacillin alone towards the disc containing Piperacillin/ Tazobactam, showing a figure of eight impression were considered as ESBL producer (as shown in Figure 1)



(Figure1)

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DISCUSSION

Extended spectrum β-lactamase (ESBL) refers to β-lactamase enzymes produced by Gram negative organisms that confer resistance against broad-spectrum β-lactam antibiotics, normally having activity against Gram negative bacilli. Examples of such antibiotics are Cefotaxime, Cefetriaxone, Ceftazidime and Aztreonam (3).The first hospital outbreak of an ESBL producing Gram negative organism was reported in Germany in 1983 (8).Within one year nosocomial outbreaks caused by a multi - drug resistant *Klebsiella* clone carrying a TEM-3 gene were described in France(9) Over the past decade, ESBL- producing *Enterobacteriaceae* have emerged as serious nosocomial pathogens throughout Europe (10). The prevalence of ESBL producing bacteria in most hospitals remains unknown in spite of numerous reports of nosocomials outbreaks of infection due to these organisms. Important ESBL producing Gram negative bacilli include *Klebsiella pneumoniae, E.coli, Proteus mirabilis, Enterobacter sp., Citrobacter freundii, Pseudomonas aeruginosa, and Acinetobacter and Stenotrophomonas maltophila* (11). The percentage of isolates expressing ESBL production is variable although a recent study from the United States reported 83 ESBL-producing isolates from 906 consecutive isolates of *Enterobacteriaceae* over a 20 week period. *Klebsiella pneumoniae* and *E.coli* were the most frequently associated with ESBL-production in the study (11). In national surveillance program conducted in 1996 in Argentina, resistance to extended spectrum Cephalosporins was shown in 48%, 26% and 8% of *Klebsiella pneumoniae, Proteus mirabilis* and *E.coli* isolates respectively (12). In a study carried out in All India Institute of Medical Sciences, New Delhi during March – June 2001, out of the 678 isolates tested 68% were ESBL producers among all Gram negative bacilli (13). In our study the prevalence of ESBL-producing *Proteus sp*. in raw milk and milk products was found to be 11% .The relative frequency of ESBL-producing *Proteus sp* .in our study is similar to frequency reported from 9.5% in the U.S. (9.5%) (15-16).

CONCLUSION

The resistance to β-lactam antimicrobial agents among Gram negative bacilli is on the increase in our setup. Laboratories can detect ESBL production by simple technique (Double disc diffusion method). Bacterial strains are resistant to most classes of antibiotics will continue to emerge unless the inappropriate use of these drugs is curtailed. Clinicians should consider ESBL production as a possibility in case of treatment failure with β-lactam antimicrobials.

REFRENCES

1. Wood AJ. Antimicrobial-drug resistance. N Eng J Med 1996; 335: 1445-53.
2. D'Agata FEMC. Antibiotic resistance and exposure to different generation cephalosporin. N Eng J Med 2000; 28: 2678-81.
3. Fridkin SK, Steward CD, Edwards JR, Pryor ER, McGowan JE Jr, Archidbald LK, et al. Surveillance of antimicrobial resistance in United States hospitals: project ICARE phase2. Clin Infect Dis 1999; 29(2):245-52.
4. Pfaller MA, Jones RN. MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) results from the Americas: resistance implications in the treatment of serious infections. J Antimicrob Chemotherapy 2000; 46(suppl):25-37.
5. Goosens H. MYSTIC (Meropenem Yearly Susceptibility Test Information Collection)

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results from Europe: Comparison of antibiotic susceptibilities between countries and centre types. J Antimicrob Chemother 2000; 46(suppl):39-52.

6. Hsueh PR, Chen ML, Sun CC, Pan HJ, Yang LS, Ho SW et al. Emergence of antimicrobial drug resistance of major pathogens causing nosocomial infections at a university hospital in Taiwan 1981-1999. Emerg Infect Dis 2002; 8(1):63-8.
7. Jarlier V, Nicolas M, Fournier G, Philippon A. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. Rev Infect Dis 1998; 10(4):867-78.
8. Knothe H,Shah P,Kremery V,Antal M, Mitsuhashi S. Transferable resistance to cefotaxime , cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens* infection 1983;11(6):315-7.
9. Brun-Buisson C, Legrand P, Philippon A, Montravers F, Ansquer M, Duval J et al. Transferable enzymatic resistance to third generation Cephalosporins during a nosocomial outbreak of multi drug resistant *Klebsiella pneumoniae*. Lancet 1987; 8(2):302-6.
10. Livermore DM, Yuan M. Antibiotic resistance and production of extended spectrum β-lactamases amongst Antimicrob Chemother 1996; 38(3):409-24.
11. Coudron PE, Moland ES, Sanders CC. Occurrence and Detection of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae* at a Veterans Medical Centre: Seek and you may find. J Clin Microbiol 1997; 35(10)2593-7.
12. Bantar C, Famiglietti A, Goldberg M. Three-year surveillance study of nosocomial bacterial resistance in Argentina. The Antimicrobial committee; and the National Surveillance Program (SIR) Participants Group. Int J Infect Dis 2000; 4(2):85-90.
13. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum β-lactamase producing Gram negative bacteria in a tertiary care hospital. Indian J Med Res 2002; 115:153-7.
14. Sirot Dl, Goldstein FW, Soussy CJ, Courtieu Al, Husson MO, Lemozy J, Meyran M, Morel C, Perez R, and Quentin-Noury C,1992. Resistance to cefotaxime and seven other β-lactams in members of the family *Enterobacteriaceae*: a 3-year survey in France. Antimicrob Agents Chemother 36: 1677-1681.
15. DeChamps C, Bonnet R, Sirot D, Chanal C, Sirot J.2000.Clinical relevance of *Proteus mirabilis* in hospital patients: a two-year survey. J Antimicrob Chemotherap 45: 537-539.
16. Saurina G, Quale JM, Manikal VM, Oydina E, and Landmam D.2000. Antimicrobial resistance in *Enterobacteriaceae* in Brooklyn, NY: epidemiology and relation to antibiotic usage patterns. J Antimicrob Chemother 45: 895-898.
17. Spanu T, Luzzaro F, Perilli M, Toniolo A, Amicosante G, and Fadda G. 2000. Resistance profile of *Enterobacteriaceae* showing synergy between clavulanate and β-lactams: an Italian survey. Clin Microbiol Infect 6(S1):79.
18. Luzzaro F, Perilli M, Amicosante G, Lombardi G, Belloni R, Zollo A, Bianchi C and Toniolo A.2001. Properties of multidrug-resistant, ESBL-producing *Proteus mirabilis* isolates and possible role of β-lactam/ β-lactamase inhibitor combinations. Int J Antimicrob Agents 17: 131-135.