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***Research Article***

# PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING PATHOGENS IN RAW MILK SAMPLES COLLECTED FROM AIZAWL TOWN, MIZORAM

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## ABSTRACT
Cow Milk is nutritious and essential food for human beings and also serves as good medium for microbial growth and contamination. Contaminated milk reduces the chances of high quality production of milk and milk based products and thereby, it affects our economy badly. Twenty five raw milk samples were collected from different cattle farms from different places of the Aizawl Town during October, 2013 to February 2014. Out of 25 samples collected, only 20% of raw milk was found in the category of Good quality, the majority of the samples i.e. 48% was in fair quality category and 32% was in the poor category. Totally 35 bacteria were isolated of 10 different genera, which includes *Staphylococcus, Streptococcus, Escherichia coli, Klebsiella, Salmonella* and others. Among the bacterial isolates, the dominant bacterial flora is *Klebsiella* species followed by *Salmonella, Citrobacter, Staphylococcus, Micrococcus* and so on. Multiple Drug Resistance (MDR) was mainly found in the genus *Klebsiella, Salmonella, Proteus, Citrobacter* and *Staphylococcus* species. Almost all the genus were showing resistance to common antibiotics like Ampicillin, Ciprofloxacin, Norfloxacin but very less to Amikacin, Meropenem and Imepenem drugs. All the gram negative bacilli were tested for Extended Spectrum β Lactamase (ESBL) enzyme production, which is an enzyme, produced by some bacteria and are responsible for their resistance to beta- lactam antibiotics like penicillins, Cephamycins and Carbapenems. The emergence of drug resistance is one of the most serious health problems in developing countries like India. In this study, the high antibiotic resistance rate (57.14%), numerous resistance pattern and high ESBL producing pathogens (61.54%) were found prevalent in the raw milk samples. Thus the material in this study gives a more reliable picture of the resistance levels that can be expected in most Enterobacteriaceae on raw milk samples in Mizoram, North East India.

***Keywords:*** *Aizawl, Antibiotic Resistance, Milk Pathogens, Public Health, Raw Milk Quality*

## INTRODUCTION
Milk is considered as nature's single most complete food Moreover; its high nutritive value makes it an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic production and storage at ambient temperatures (Kim *et al*., 1983; OECD, 2005). Milk is complex mixture of fat, protein, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water, make it a complete diet (Haug *et al*., 2007). Contaminated raw milk can be a source of harmful bacteria. Raw or processed milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product or infections / intoxications in consumers (Murinda *et al*., 2004; Oliver *et al*., 2005). Microbes may gain entry into raw milk directly from dairy cows experiencing sub clinical or clinical mastitis (Rodojcic *et al.*, 1991), from the farm environment particularly the water source (Eberhart, 1977) and utensils used for the storage of milk on farm or during transportation (Freedman, 1977).

In India raw milk is traditionally consumed at the small farms where it is produced or fermented into different products. During scaling up, the hygienic aspects are not always sufficiently considered. The risk of contaminated and pathogen containing products could therefore be even greater than when the milk is processed at household level (FAO and WHO, 1997). The delayed time of milking process performance and low hygienic conditions were possible to grow the microorganisms. The contamination

leads to pathogenic microbes grows well the milking media. Pathogens that have been involved in food borne outbreaks include *Salmonella*, *Staphylococcus aureus* and *E. coli*. The presence of these pathogenic bacteria in milk emerged as major public health concerns, especially for those individuals who still drink raw milk (Riser, 1998). Keeping fresh milk at an elevated temperature together with unhygienic practices in the milking process may also result in microbiologically inferior quality. Apparently, these are common practices for small-scale Asian produce fresh milk and sell it to consumers (Chye *et al.,* 1994).

Antimicrobials have been used frequently as a conventional measure to prevention and control diseases in dairy farming. Especially in mastitis control programs, more and more antibiotics were applied even without any clinical symptoms in dairy cattle herds. However, long term in-feed use of antibiotics on dairy farms has lead to the alarming increase of antibiotic resistant bacteria and which has become a public health issue worldwide, e.g., Methicillin-resistant *S. aureus* (MRSA) from raw milk and environmental samples constitutes a great threat to food safety (Aumaitre, 1999).

Risk-based approaches offer a new way of managing food safety in developing countries. Not only are they more effective at decreasing risks, but they can also be a bridge between food safety and livelihood concerns. In view of the growing public awareness about food safety and quality, knowledge of the microbial and chemical composition of milk is of great significance for further development of its hygienic processing into high quality consumer products. Until now, information on such aspects is scant and scattered. Thus this study was carried out to investigate the microbiological quality and safety of raw cow milk in Mizoram, a North- eastern state of India.

## MATERIALS AND METHODS

**Collection of Samples:** A total 25 raw cow milk samples were collected from 7 dairy farmers who send their milk-to-milk centers (MC) in Aizawl, Mizoram. Also 5 commercial milk products available in the Aizawl market were included in the current study for quality control purpose. Samples were collected in the early morning. Approximately 50-100ml milk was aseptically sampled from containers (buckets or Churns) of bulk milk from each individual farmer into a sterile bottle or screw cap tubes. It was collected immediately after milking using hand or machine in to bulk milk containers at ambient temperature (28-30$^{o}$C). Samples were transported to the laboratory in a cool box at less than 4$^{o}$C within 1 h of collection and tested immediately upon arrival; the processing was not delayed more than 3 hours.

**Total Viable Count (TVC) and Microbiological Analysis:** Serial dilutions of samples were made up to $10^{-7}$ in double distilled water. Samples were plated in duplicate using pour plate technique. 0.5ml of the diluted sample was delivered by pipette in to 19.5 ml of Nutrient agar. Plates were incubated in an inverted position at 37$^{o}$C for 24-48hrs. Total viable counts were carried out on nutrient agar. Quantitative analysis for the presence or absence of specific microorganisms was done by plating on selective media. Enumeration of total plate count, coli form, *E. coli* and *S. aureus* were carried out as described by standard methods of the American Public Health Association (APHA, 1960). Typical isolates were confirmed based on their Grams staining characters, Motility, Catalase and IMViC pattern etc. Mannitol Salt Agar and Hi-chrome MeReSa agar (Hi Media Labs, India) was used for quantitative detection of *S. aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA). And selective media like Eosin Methylene Blue (EMB) agar and Salmonella-Shigella Agar (Hi-Media, India) were used to detect the presence of E.coli and Salmonella respectively.

**Characterization of Isolates from Milk Samples:** At intervals, colonies on the incubated plates were picked and purified by repeated sub-culturing by streaking on the desired media with a sterile wire loop. The strategy consisted of picking one colony to represent every visibly different morphology on each plate. A maximum of 5 colonies were obtained per sample, which were examined microscopically for Gram's reaction and colony morphology (shape, size, colour, texture) using 24h old cultures. Motility and biochemical tests were performed. Appropriate positive and negative controls were used to make distinction between positive and false-positive reactions. Results were analyzed using Bergy's manual, and other methods for the identification (Collee *et al.,* 2011), Mackie and McCartney Practical Medical Microbiology (2011) and Mahon *et al*., (2007) Text book of Diagnostic Microbiology".

**Antibiotic Susceptibility Tests:** Briefly, the susceptibility of all the isolates against the antimicrobials was determined by Kirby-Bauer disc diffusion method in Mueller-Hinton agar (Bauer-Kirby, 1996). The inoculum was prepared at a density adjusted to a 0.5 McFarland turbidity standard solution. Commercially available antimicrobial discs (HiMedia Ltd, Mumbai, India) of Ceftazidime (CAZ 30= 30 µg), Aztreonam (AT10 = 10 µg), Nitrofurantoin (NIT200 =200 µg), Ciprofloxacin (CF10 =10 µg), Ampicillin (A10 = 10 µg), Cefixime (CFM5 = 5 µg ), Meropenem (MRP10= 10 µg), Imepenem (IPM10 =10µg), Linezolid (LZ30 =30µg), Cefoxitin (CX30 =30µg),Co- trimoxazole (CO25 = Trimethoprim 2.25µg and Sulphamethoxazole 22.75 µg )**,** Norfloxacin (NX10 =10 µg ), Cephotaxime (CE30 =30 µg), Amikacin (AK10 =10 µg) and Clindamycin (CD10 =10 µg )were placed on the inoculated agar plates and incubated in an upright position overnight at 37ºC. Sensitivity was recorded after 24 hrs of incubation by measuring the zone of inhibition formed around the antimicrobial discs. The results were expressed as Sensitive, Intermediate and Resistant by considering CLSI, 2012 guidelines.

**Statistical and Data Analysis:** Bacterial load, mean counts of isolates and ESBL producers were statistically analyzed by one way Analysis of Variance. Significant differences between treatments were determined using Turkey's multiple range test at P =0.05 with the help of GraphPad InStat software Version. 3.6.

## RESULTS AND DISCUSSION

Out of 25 raw milk samples of cows 35 bacterial isolates belongs to 10 genuses were obtained. The raw milk samples were collected from 7 dairy farmers of three different regions in Aizawl town, Mizoram. The results presented in table 1, reveals that out of 25 raw milk tested, 35 isolate of gram negative and gram positive bacteria including some pathogens were encountered. The isolates were occurred as *Klebsiella* sp. (**7;** 20%), *Salmonella* sp. (**6;** 17.14%), *Citrobacter* sp. (**5;** 14.29%), *Staphylococcus* sp., *Proteus* sp., *Micrococcus* sp., and *E.coli* (**3;** 8.57%), *Streptococcus* sp., and *Pseudomonas* sp. (**2;** 5.71%) and *Corynebacterium* sp. (**1;** 2.87%). The results of table 2 reveal that only 20% of raw milk is found in the category of good quality, the majority of samples i.e. 48% was in fair quality and 32% was in the poor quality categories. Chatterjee *et al.,* (2006) reported that the raw milk contained higher number of micro flora probably due to contamination from the animals.

**Total Viable Count (TVC):** Fresh raw cow milk collected from different farms was detected heavily contaminated by bacteria by Total viable Count (TVC). Total viable count was ranging from a least viable count $1.0x10^3$cfu/ml and the highest count of $7.72 \times 10^6$ cfu/ml, both the counts were found from Thuampui region. TVC from the Zemabawk region showed the least count of $2.8 \times 10^3$ cfu/ml and the higher count of $3.98 \times 10^6$ cfu/ml from same farm. TVC from the Zuangtui region showed the least count of $6.2 \times 10^4$ cfu/ml and the higher count of $2.31 \times 10^6$ cfu/ml from same farm (Data not Shown). Results from the analysis variance (ANOVA) suggested that there was a significant difference ($p< 0.05$) in bacterial loads between the regions.

Bacteria may enter milk through the udder and most of the organisms in raw milk are contaminants from the external surface of udder, milking utensils and handlers (Ayres *et al*., 1980). Various types of equipment and utensils, such as milking machines, pails, cans and milk churns are used in handling milk on the farm. In order to reduce contamination of milk, utensils used for milking should be rinsed, cleaned using detergent and disinfected immediately after use (FAO and WHO, 1997; Dodd and Phipps, 1994).

**Antibiotic Susceptibility Testing:** A total of 35 strains were isolated from the tested samples and assessed for susceptibility to selected common antimicrobials. Results are summarized in Table 3. All the samples contained strains showing resistance to at least two antibiotics. Resistance to Ampicillin, Cephotaxime and Clindamycin was rather common among the tested strains (82%, 60% and 57% of isolated strains, respectively) if compared to that to Meropenem (5%), Imepenem (14%) and Amikacin (8%). The antibiogram profile of different bacterial isolates indicated that Meropenem, Imepenem, Amikacin, Ciprofloxacin and Norfloxacin proved to be the most effective antimicrobials against bacterial isolates in the study (Table-3). Ampicillin was found to be least effective antibiotic against bacterial isolates. It may be due to indiscriminate and frequent use of this antibiotic in dairy animals leading to

development of antibiotic resistance. The antibiotic resistant isolates found in raw milk are harmful to cattle's and humans, it will result in therapy failure which may lead to high mortality and morbidity of cattle's as well as humans. Antimicrobial agents are used widely as food additives to improve growth and feed conversion in many types of animal operations, including poultry, swine and cattle operations. And in humans misuse of antibiotics or self medication is so common. As a result, antibiotic resistance in the bacterial communities in the intestinal tracts of domestic animals and in humans has become common (Aerestrup *et al*., 2000).

**Table 1: Percentage of Occurrence of bacterial isolates in raw milk**

| Sl. No | Name of the isolates. | Percentage of Occurrence. (%). |
|---|---|---|
| **1.** | *Staphylococcus sp.* | 03 (08.57) |
| **2.** | *Streptococcus sp.* | 02 (05.71) |
| **3.** | *Klebsiella sp.* | 07 (20) |
| **4.** | *Proteus sp.* | 03 (08.57) |
| **5.** | *Micrococcus sp.* | 03 (08.57) |
| **6.** | *Escherichia coli.* | 03 (08.57) |
| **7.** | *Citrobacter sp.* | 05 (14.29) |
| **8.** | *Pseudomonas sp.* | 02 (05.71) |
| **9.** | *Corynebacterium sp.* | 01 (02.87) |
| **10.** | *Salmonella sp.* | 06 (17.14) |
| **Total** | | 35 (100) |

**Table 2: Analysis of raw milk quality (Ref. IS: 1479-1977, PART III)**

| Sl. No | Total No. of Samples | Quality of tested samples (%) | Quality Grade of Milk |
|---|---|---|---|
| 1. | | 00 | Very Good |
| 2. | 25 | 05 (20) | Good |
| 3. | | 12 (48) | Fair |
| 4. | | 08 (32) | Poor |
| Total | | 25 (100) | |

**Table 3: Multiple Drug Resistance (MDR) patterns of isolates against the selected antibiotics**

| Sl. No. | Organisms (n= No of isolates) | CAZ 30 | AT 30 | NIT 200 | CF 10 | A 10 | CFM 5 | MRP 10 | IPM 10 | LZ 30 | CX 30 | CO 25 | NX 10 | CE 30 | AK 10 | CD 10 | MDR (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *Staphylococcus* sp. (n= 3) | 2 (66) | 1 (33) | 1 (33) | 1 (33) | 3 (100) | 0 | 0 | 0 | 2 (66) | 2 (66) | 0 | 1 (33) | 2 (66) | 0 | 2 (66) | 2 (66.66) |
| 2. | *Streptococcus* sp. (n= 2) | 1 (50) | 1 (50) | 0 | 0 | 2 (100) | 1 (50) | 0 | 0 | 1 (50) | 1 (50) | 1 (50) | 0 | 0 | 0 | 1 (50) | 1 (50) |
| 3. | *Klebsiella* sp. (n=7) | 4 (57) | 4 (57) | 6 (86) | 1 (14) | 7 (100) | 6 (86) | 0 | 0 | 7 (100) | 6 (86) | 6 (86) | 1 (14) | 6 (86) | 1 (14) | 7 (100) | 4 (57.14) |
| 4. | *Proteus* sp. (n= 3) | 3 (100) | 3 (100) | 2 (66) | 0 | 3 (100) | 2 (66) | 1 (33) | 1 (33) | 3 (100) | 3 (100) | 1 (33) | 2 (66) | 3 (33) | 0 | 3 (100) | 3 (100) |
| 5. | *Micrococcus* sp. (n=3) | 2 (66) | 1 (33) | 1 (33) | 1 (33) | 2 (66) | 1 (33) | 0 | 0 | 1 (33) | 0 | 0 | 1 (33) | 2 (66) | 0 | 0 | 1 (33.33) |
| 6. | *E.coli* (n= 3) | 2 (66) | 1 (33) | 1 (33) | 2 (66) | 2 (66) | 1 (33) | 0 | 0 | 1 (33) | 1 (66) | 1 (33) | 2 (66) | 2 (66) | 0 | 1 (33) | 1(33.33) |
| 7. | *Citrobacter* sp. (n=5) | 2 (40) | 2 (40) | 1 (20) | 3 (60) | 4 (80) | 2 (40) | 1 (20) | 0 | 1 (20) | 2 (40) | 2 (40) | 1 (20) | 2 (40) | 1 (20) | 2 (40) | 2 (40) |
| 8. | *Pseudomonas* sp. (n=2) | 1 (50) | 1 (50) | 0 | 1 (50) | 2 (100) | 1 (50) | 0 | 0 | 0 | 1 (50) | 1 (50) | 1 (50) | 0 | 0 | 1 (50) | 1 (50) |
| 9. | *Corynebacterium* sp. (n=1) | 0 | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 0 | 1 (100) | 0 |
| 10. | *Salmonella* sp. (n= 6) | 2 (33) | 3 (50) | 4 (66) | 2 (33) | 5 (83) | 1 (16) | 0 | 4 (66) | 3 (50) | 2 (33) | 2 (33) | 3 (50) | 4 (66) | 1 (16) | 2 (33) | 3(50) |
| Total (n = 35) (% resistance) | | 19 (54.29) | 17 (48.57) | 16 (45.71) | 12 (34.29) | 29 (82.86) | 15 (42.86) | 02 (05.71) | 05 (14.29) | 19 (54.29) | 19 (54.29) | 15 (42.86) | 12 (34.29) | 21 (60) | 03 (08.57) | 20 (57.14) | 18 (51.43) |

**Table 4: Prevalence of ESBL producing Gram negative bacillus (GNB's) on raw Milk of Aizawl town**

| Sl. No. | Organisms (Gram Negative Bacillus) | % of isolation | % of ESBL |
|---|---|---|---|
| 1. | ***E.coli*** | 3 (11.54) | 2 (7.69) |
| 2. | ***Klebsiella* sp.** | 7(26.92) | 5 (19.23) |
| 3. | ***Proteus* sp.** | 3(11.53) | 2 (7.69) |
| 4. | ***Pseudomonas* sp.** | 2(7.69) | 1 (3.85) |
| 5. | ***Citrobacter* sp.** | 5(19.23) | 3 (11.54) |
| 6. | ***Salmonella* sp.** | 6(7.69) | 3 (11.54) |
| Total (n*=26 ) | | 26 (100%) | 16 (61.54) |
| SD Values | | 07.563 | 5.254 |
| SEM | | 03.088 | 02.145 |
| p. Value (One tail) | | 0.0379 | |

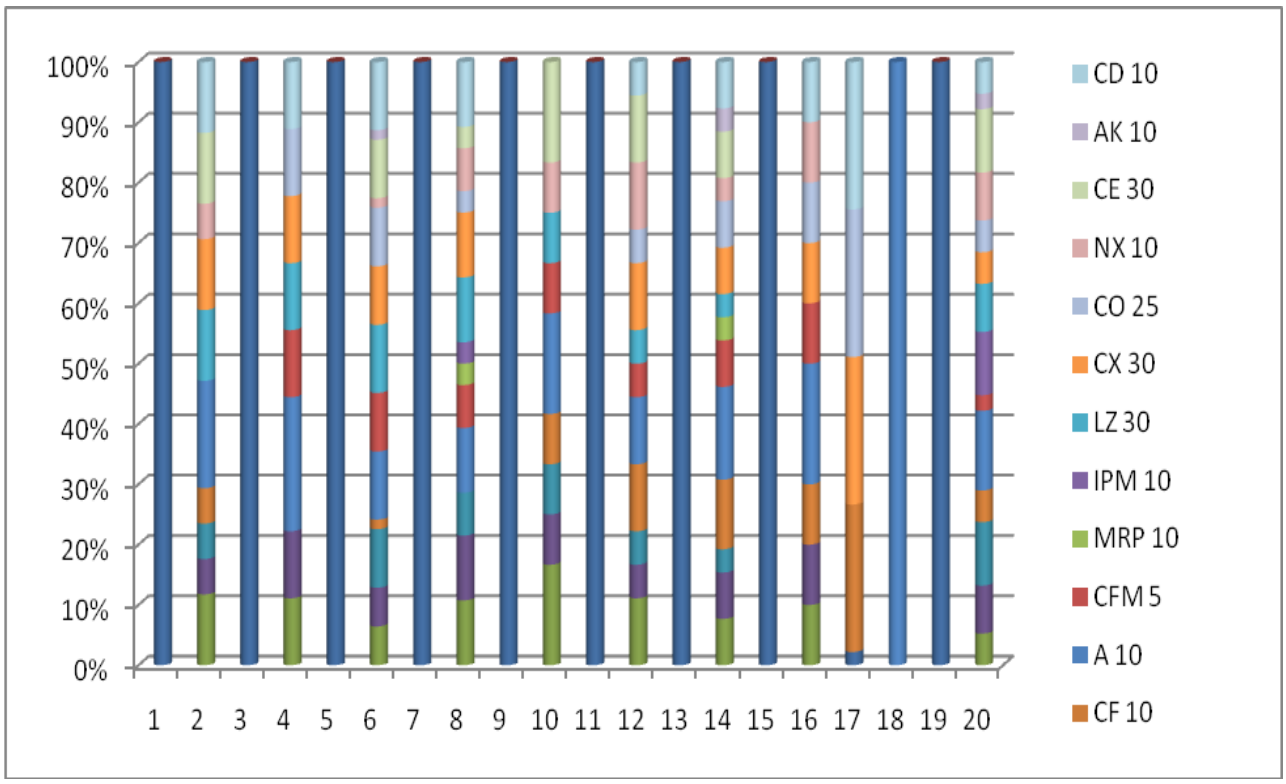


**Figure 1: Antibiogram and MDR pattern of isolates**

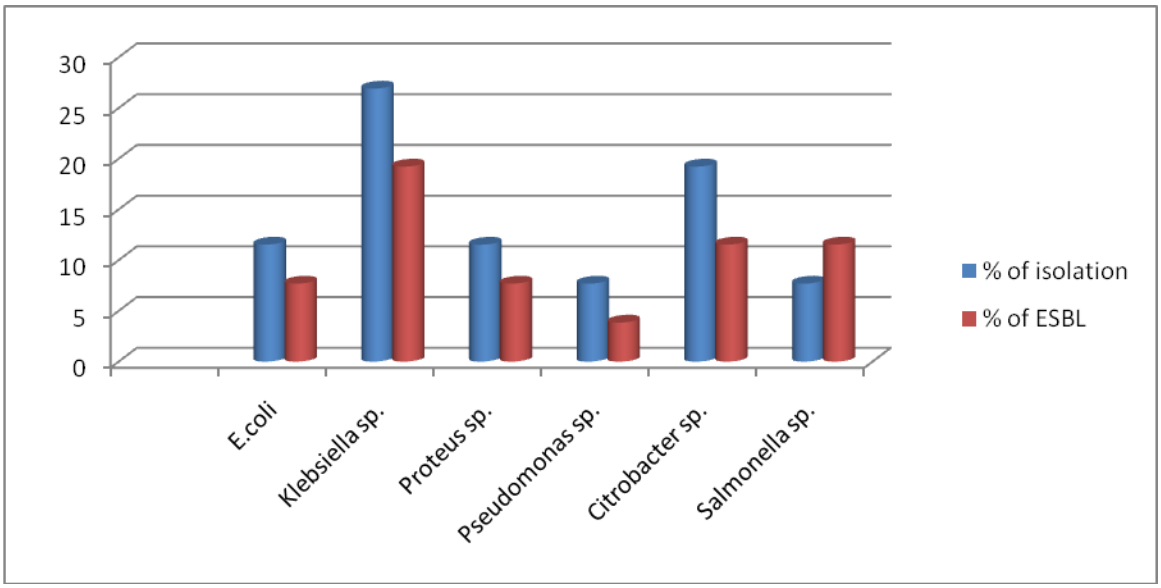


**Figure 2: Percentage distribution and ESBL producing isolates**

The emergence of drug resistance is one of the most serious health problems in developing countries like India. In this study, the high antibiotic resistance rate (51.43%), numerous resistance patterns and high ESBL producing pathogens (61.54%) were found prevalent in the vegetables. Thus the material in this study gives a more reliable picture of the resistance levels that can be expected in most Enterobacteriaceae on vegetable in North East India.

Multi drug resistance (MDR) pattern was observed in most of the strains isolated. Among the 35 strains isolated 18 (51.43%) were Multi Drug Resistant (MDR) strains which are very high compared to previous report by the authors in UTI patients from the same study area (Karuppasamy and Lalsanglura, 2012). Of those, except *Corynebacterium* spp. all other strains were found resistant to more than 5 drugs at least. Drug resistance percentage is increased in comparison to the previous study, 0% strains were resistant to Meropenem and Imepenem which is now showing 05.71% and 14.29% respectively in this study.

**Extended Spectrum β- Lactamases:** Extended Spectrum β Lactamases (ESBLs) are a group of enzymes that have the common property of providing resistance to extended-spectrum β lactam antibiotics such as Oxyimino cephalosporins (e.g. cefotaxime, ceftazidime, ceftriaxone, cefepime and cefpirome), as well to aztreonam an oxyimino monobactam, Cephamycins (Cefoxitin and cefotetan) and Carbapenems (Imepenem and Meropenem) (Oreste, 2003).

All the gram negative isolates have been tested for the extended spectrum β- lactamase enzymes (ESBL) by standard bacteriological double diffusion synergy test (DDST) methods (CLSI, 2012). Of all the members of Enterobacteriaceae Gram Negative Bacilli (GNB) isolates *Klebsiella* spp. was found to be the highest ESBL producer that 19.23%, followed by *Citrobacter* and *Salmonella* (11.54%). But *Proteus* spp. (7.69%), *E.coli* (7.69%) and *Pseudomonas* sp. (3.85%) contributes very less percentage of ESBL productions (Table.4). In this study 16 (61.54%) strains out of 26 GNB found to be positive for the ESBLs double disk synergy test. This indicates that the resistance genes might have transferred to the pathogens of cattle's from human pathogens or vice versa. This high prevalence of ESBL producing, Multi Drug Resistant bacteria on the freshly collected raw milk is representing a high potential health risk to human population consumes or exposed to these raw milk samples of the current study locations.

***Conclusion***

In conclusion, most of the raw milk samples analyzed in this study contained the declared bacterial species in sufficient amount. Moreover, specific antibiotic resistance found in all isolated strains is of great concern. This might be the consequence of an extensive use of antimicrobials which has created a selective pressure for point mutations and acquisition of Mobile Genetic Elements (MGE) encoding

antimicrobial resistance. One limit of our study was the lack of genetic analysis of antibiotic resistance. Further analyses will aim to investigate the real safety of raw milk consumers and to better understand the mechanisms behind the observed antibiotic resistance, in order to avoid serious health issues for human mainly infants and children.

## REFERENCES

**Aarestrup FM, Agerso Y, Smidt PG, Madsen M and Jensen LB (2000).** Comparison of antimicrobial resistance phenotypes and resistance genes in E. *faecalis* and *E. faecium* from humans in the community, broilers and pigs in Denmark. *Diagnostic Microbiology and Infectious Diseases* **37** 127-137.

**APHA (1960).** Standard Methods for the Examination of Water and Waste Water. In: *American Public Health Association,* edited by Eaton AE, Clesceri LS and Greenberg AE (United Book Press Inc.) Maryland.

**Aumaître A (1999).** Quality and Safety of Animal Products. *Livestock Production Science* **59** 113-124.

**Ayres JC, Mundt JO and Sandinc WE (1980).** *Microbiology of Foods* (W. H Freeman) San Francisco 42-56.

**Bauer AW, Kirby MDK, Sherries JC and Truck M (1966).** Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* **45** 493-496.

**Chatterjee SN, Bhattacharjee I, Chatterjee SK and Chandra G (2006).** Microbiological examination of milk in Tarakeswar, India with special reference to coli -forms. *African Journal of Biotechnology* **5** 1383 – 1385.

**Chye FY, Aminah A and Khan AM (1994).** Microbiological quality of milk produced by three types of milking methods. *In Proceeding of the Fifth ASEAN Food Conferences*, Kuala Lumpur, Malaysia 26-29.

**Clinical Laboratory Standards Institute M100-S22 (2012).** Clinical and Laboratory Standards Institute: performance standards for antimicrobial susceptibility testing, 22nd Informational supplement (M100- S22). Clinical and Laboratory Standards Institute **31**(1), Villanova. Pa. USA.

**Collee JG, Fraser AG, Marmion BP and Simmons A (2011).** *Mackie & McCartney- Practical Medical Microbiology*, 14th edition (Churchill Livingston).

**Determinative Bacteriology (1994).** *Bergey's Manual of Determinative Bacteriology* 9th Edition (Williams and Wilkins Co.) Baltimore.

**Dodd FH and Phipps RH (1994).** Dairy management and health. In: *Milk Production in Developing Countries,* edited by Smith AJ, Centre for Tropical Veterinary Medicine, University of Edinburgh, Scotland, UK 258-271.

**Eberhart RJ (1977).** Coli form mastitis. *Journal of American Veterinary Association* **170** 1160-1163.

**FAO and WHO (1997).** General requirements (food hygiene). *Codex Alimentarius,* Food and Agriculture Organization, Rome **1B**(suppl).

**Freedman B (1977).** Milk quality. In: *Sanitarians Handbook: Theory and Administrative Practice for Environmental Health*, fourth edition (Peerless publishing) 564-589 New Oreleans, USA.

**Haug A, Hostmark AT and Harstad OM (2007).** Bovine milk in human nutrition – a review. *Lipids in Health and Disease* **6** 25.

**Karuppasamy C and Lalsanglura Ralte (2012).** Resistance of the pathogens causing Urinary Tract Infections against antibiotics. *Bioinfolet* **9**(3) 348 – 354.

**Kim H, Hardy J, Novak G, Ramet JP and Weber F (1983).** Off-tastes in raw and reconstituted milk. FAO *Animal Production and Health* **35** 2.

**Mahon CR, Lehman DC and Manuselis G (2007).** *Textbook of Diagnostic Microbiology,* 3rd edition, Saunders, Missouri.

**Murinda SE, Nguyen LT, Man HM and Almedia RA (2004).** Detection of sorbitol negative and sorbitol positive shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter* jejuni and *Salmonella* species in dairy farm environments. *Food borne Pathogens and Disease* **1** 97-104.

**OECD (2005).** Dairy policy reform and trade liberalization. *Organization for Economic Co-operation and Development* (OECD publishing) 98.

**Oliver SP, Jayarao BM and Almedia RA (2005).** Food borne pathogens in milk and the dairy environment food safety and public health implications. *Food Borne Pathogens and Disease* **2** 1115-1129.

**Oreste AM (2003).** Bacteria versus Antibacterial agents, an integrated approach. *American Society of Microbiology* (ASM Press) 172-182.

**Riser ET (1998).** Public health concerns. In: edited by Marth EH and Steele JL *Applied Dairy Microbiology* (Marcel Dekker, Inc) New York 263-403.

**Rodojcic-Prodaova D and Necev T (1991).** Most common agents of subclinical mastitis in cows on private and communal farms in the republic of Macedonia vet glasnik **45** 745-747.

**IS 1479-3. (1977).** India Standard Methods of Test for Dairy Industry, Part-III: Bacteriological Analysis of Milk. Reaffirmed, 2003. Bureau of Indian Standards. New Delhi.