

ANTIBIOTIC RESISTANT OF PATHOGENIC BACTERIA ISOLATED FROM MILK AND MILK PRODUCTS IN AKOLA CITY OF MAHARASHTRA

JAVED KHAN^{a1}, M. MUSADDIQ^b AND G. N. BUDHLANI^c

^{ab}G. Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola, M. S., India

^cAadarsh Mahavidyalaya, Dhamangaon, M. S., India

ABSTRACT

Antimicrobial resistance is said to currently be the greatest challenge to the effective treatment of infections globally. This study evaluated the risks of antimicrobial resistant microbes associated with different Milk and Milk Products (Raw Milk, Packaged Milk, Curd, Khoya, Paneer) collected from local market of Akola. The Common isolates identified were *E. coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Shigella* spp. Antibiotic susceptibility tests indicated that all (100%) isolates were resistant to Ampicillin (AMP), Tetracycline (TET), Chloramphenicol (CHL), Gentamycin (GEN), Cotrimoxazole (COT), Ceftriaxone (CTR), Vancomycin (VAN) and Methycillin (MET), Imipenem and Meropenem. *Staphylococcus aureus* was found that approximately 94% resistance and *Escherichia coli* was 85% resistant to all above antibiotics similarly 93% resistance for antibiotics was shown by *Salmonella typhi* and 87% for *Shigella* spp. The study demonstrated that Milk and Milk products sold in Akola is a potential hazard of pathogenic food borne bacteria as well as antimicrobial resistant bacteria that may have public health implications. There is the need for some additional food safety measures to be applied before the consumption of milk..

KEYWORDS : Antibiotic Agents, Resistant Bacteria, Milk and Milk Products

Milk is an essential part of daily diet for the growing children and expectant mothers. Milk, is a major constituent of the diet, its quality assurance is considered essential to the welfare of a community. Milk is nutritious food for human beings, also serves as a good medium for the growth of many microorganisms, especially *Lactobacillus*, *Streptococcus*, *Staphylococcus* and *Micrococcus* sp. Bacterial contamination of raw Milk can originate from different sources from animals such as air, Milking equipment, feed, soil, feces and grass (Torkar & Teger, 2008).

Milk is supposed to constitute a complex ecosystem for various microorganisms including bacteria. Milk products like cheese and curd are widely consumed and market for them has existed in many parts of the world for many generations. There is an increase demand by the consumer for high quality natural food, free from artificial preservatives, and contaminating microorganisms. Contamination of Milk and Milk products, with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions. (Priyanka Singh and Alka Prakash, 2008).

The uncontrolled application of antimicrobials in the environment is leading to a constant increase in the rate of antimicrobial resistance. *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Shigella* spp. Can rapidly acquire resistance to a broad range of

antimicrobials, thereby posing a major concern in the treatment of infections. Studying antimicrobial resistance in humans and animals is important for detecting changing patterns of resistance, implementing control measures on the use of antimicrobial agents and preventing the spread of multidrug-resistant strains of bacteria. (Zouhairiet al., 2010).

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola. (Maharashtra). During the period from October 2012 to May 2013.

Sample Collection

100 Samples of Milk and Milk product like Raw Milk (25), Packaged Milk (25), Curd (20), Khoa (20), and Paneer (10) were collected from the market. Milk and Milk product samples were directly transported to the laboratory in ice box. They were stored in refrigerator and analyzed within 24 hours.

Isolation and Identification of Bacteria

A portion (1 g or 1 ml) from each sample was taken aseptically and diluted in 9 ml sterile distilled water. The diluted sample was streak inoculated on sterile Nutrient Agar and incubated at 37°C for 24 hours. After incubation period all the colonies undertaken for Gram Staining as per the results of Gram Staining respected colonies were

¹Corresponding author

inoculated on Sterile Selective media such Mannitol Salt agar (M. S. A) For *Staphylococcus aureus*, Eosin Methylene Blue (E. M. B) for *Escherichia coli*, Xylose lysine deoxycholate agar (X. L. D) for *Salmonella typhi* and for *Shigella* spp, *Salmonella-Shigella* agar (S. S. agar) was used. Identification was one by Biochemical characteristics.

Antibacterial Susceptibility Testing

Antibiotic susceptibility screening was done as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). Kirby- Bauer's disc diffusion technique was adapted for antibiogram. The antibiotic discs and Mueller- Hinton Agar were purchased from Hi-Media, Mumbai. The plates were prepared as per the manufacturer's instructions and checked for sterility by

incubating the plates overnight at 37°C. The antibiotics discs were kept at room temperature for 1 hour before use.

RESULTS AND DISCUSSION

Pathogenic bacteria in Milk have been a major factor for public health concern since the early days of the dairy industry. Many diseases are transmissible via Milk products. Traditionally raw or unpasteurized Milk has been a major vehicle for transmission of pathogens. The Health of dairy herd and milking conditions basically determine the milk quality. Another source of contamination by microorganisms is unclean teats. The use of unclean milking and transport equipments also contributed to the poor hygienic quality (Parekh and Subhash, 2008).

Table 1 : Distribution of Various Milk and Milk Products Samples on the Basis of Bacterial Pathogen

Sr. No	Types of Sample	No. of Samples	Isolated bacteria from Milk and Milk Products			
			<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>Shigella spp</i>
1.	Raw Milk	25	20	15	10	03
2.	Packed Milk	25	10	10	02	Nil
3.	Curd	20	15	20	08	05
4.	Khoa	20	18	15	10	08
5.	Paneer	10	05	08	05	05

Table 2 : Antibiotic Resistance of Identified Isolates to 8 Antimicrobial Agents

Sr. No.	Bacterial isolates	No. of Isolates	Antibiotic Resistance (%)									
			AMP	TET	CHL	GEN	COT	CTR	VAN	MET	IMIPENEM	MEROPENEM
1)	<i>S. aureus</i>	68	98%	95%	98%	94%	98%	96%	85%	90%	Sensitive	Sensitive
2)	<i>E. coli</i>	68	98%	92%	95%	80%	95%	95%	75%	82%	Sensitive	Sensitive
3)	<i>S. typhi</i>	35	98%	95%	98%	90%	98%	95%	80%	90%	Sensitive	Sensitive
4)	<i>Shigella spp</i>	21	95%	95%	95%	85%	95%	98%	60%	75%	Sensitive	Sensitive

Ampicillin(AMP), Tetracycline(TET), Chloramphenicol(CHL), Gentamycin(GEN), Cotrimoxazole(COT), Ceftriaxone (CTR), Vancomycin (VAN) and Methycillin (MET).

Table 3 : Antibiotic Resistance Pattern of Pathogenic Bacteria (Average Wise)

Sr.No.	Bacterial isolates	Number of Bacteria Isolated/Percentage		
		Total	Resistant bacteria	Susceptible bacteria
1)	<i>S. aureus</i>	73	94%	6%
2)	<i>E. coli</i>	47	85%	15%
3)	<i>S. typhi</i>	68	93%	7%
4)	<i>Shigella spp</i>	71	87%	13%

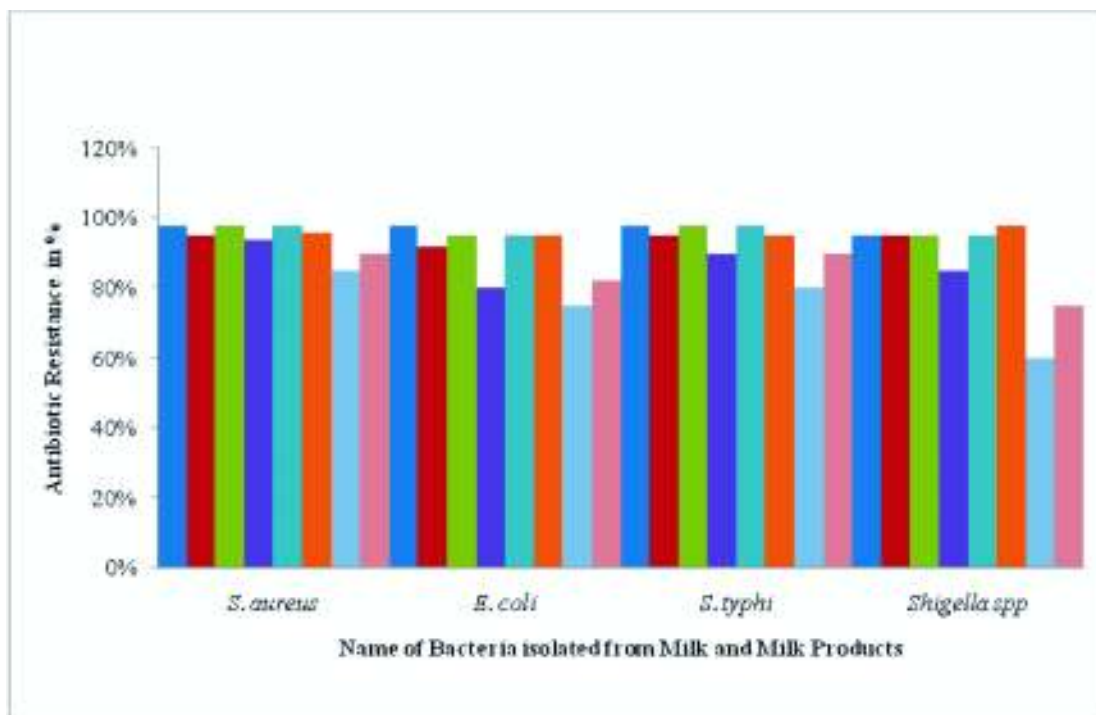


Figure 1 : Antibiotic Resistance Pattern of Pathogenic Bacteria Isolated from Milk and Milk Products.

DISCUSSION

The present study was undertaken to detect the antibacterial resistance of pathogenic bacteria isolated from Milk and Milk products. The presence of pathogenic bacteria from Milk and Milk products will show the unhygienic practices and bad quality of Milk and Milk products also detection of Multidrug resistant bacteria from this source should give idea about hazards of Milk and Milk products in our market.

In this study bacteria isolated from Milk and Milk product was subjected for Antibacterial resistance pattern of above all Pathogenic bacteria against Ampicillin(AMP), Tetracycline (TET), Chloramphenico (CHL), Gentamycin (GEN), Cotrimoxazole (COT), Ceftriaxone (CTR), Vancomycin (VAN) and Methycillin (MET) antibiotics, Imipenem and Meropenem. The results was very unexpected the *Staphylococcus aureus* was found that approximately 94% resistance and *Escherichia coli* was 85% resistant to all above antibiotics similarly 93% resistance for antibiotics was shown by *Salmonella typhi* and 87% for *Shigella spp*. According to study of (Ebtsham et al., 2009) it was reported that all the bacteria isolated

from Milk and Milk products *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* was found to be 68% resistance to the various antibiotics. According to them the highest resistance shown by bacteria towards Chloromphenicol but according to investigation of (Sharma et al., 2011) it was found that *S. aureus* was highly resist to vancomycin. On the other hand several isolates were found susceptible to the Ofloxacin, Ampicillin, Tetracycline Oxacillin, Streptomycin, Sulphafurazole and Ciprofloxacin.

(Mahami et al., 2011) it was observed that (100%) isolates *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella spp* were multi-resistant to Ampicillin, Tetracycline, Chloramphenicol, Gentamycin, Cotrimoxazole, Cefuroxime and cefotaxime. Ceftriaxime was the most effective antimicrobial but even then, 90.57% of isolates were resistant to it.

This study suggested that more efforts are needed to enhance and promote farms and sale points of milk by using screening confirmatory tests at sales points and farms. Moreover, the ministries concerned should adopt comprehensive strategy for ensuring a safe supply of good

quality milk. These strategies should include promoting knowledge of farmers' standards through training and extension and the adoption of grading and quality testing of milk. Ultimately, the milk testing programs should become component of the quality process that should focus on production of high quality milk.

REFERENCES

- Bramley A. J. and McKinnon C. H., 1990. "The Microbiology of Raw Milk". In: Dairy microbiology, I. Robinson RK (Ed.), London, NY, Elsevier Applied Sci., : 171.
- O. Zouhairi, I. Saleh, N. Alwan, I. Toufeili, E. Barbour and S. Harakeh, 2010. Antimicrobial resistance of Staphylococcus species isolated from Lebanese dairy-based products". Eastern Mediterranean Health Journal EMHJ, Vol. 16 No.12.
- Parekh T. S. and Subhash R., 2008. "Molecular and bacteriological examination of Milk from different milch animals with special references to Coliforms". Curr. Res. Bacteriol. 1(2), 56-63.
- Priyanka Singh and Alka Prakash, 2008. "Isolation of Escherichia coli, Staphylococcus aureus and Listeria monocytogenes from Milk products sold under market conditions at Agra region". Acta agriculturae Slovenica, 1: 83-88.
- Torkar K. G. and Teger S. G., 2008. "The Microbiological quality of raw Milk after introducing the two day's Milk collecting system". Acta Agri. Slovenica. 92(1), 61-74.
- Ibtisam E. M. El Zubeir and O.A.O. El Owni, 2009. "Antimicrobial resistance of bacteria associated with Raw milk contaminated by chemical preservatives" World Journal of Dairy & Food Sciences 4(1): 65-69, 2009 ISSN 1817-308X.
- Mahami T., Odonkor S., Yaro M., Adu-Gyamfi A., 2011. "Prevalence of antibiotic resistant bacteria in milk sold in Accra" International Research Journal of Microbiology 2(4): 126-132, May 2011.