



## **Resistant Pattern of Therapeutics Antimicrobial Challenged on *Pseudomonas aeruginosa* Bacterium Isolated from Marketed Raw Buffalo Milk**

**N. M. Patel<sup>1</sup>, R. Kumar<sup>1\*</sup>, A. P. Suthar<sup>1</sup>, C. V. Savalia<sup>1</sup>, D. N. Desai<sup>2</sup> and I. H. Kalyani<sup>2</sup>**

<sup>1</sup>*Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, Navsari - 396 450, Gujarat, India.*

<sup>2</sup>*Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, Navsari - 396 450, Gujarat, India.*

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors NMP and RK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors APS, DND and CVS managed the analyses of the study. Author IHK managed the literature searches. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/EJNFS/2019/v9i430085

#### Editor(s):

(1) Dr. Rasha Mousa Ahmed Mousa, Associate Professor, Department of Biochemistry, University of Jeddah, Jeddah, Saudi Arabia.

#### Reviewers:

(1) Md. Tanvir Rahman, Bangladesh Agricultural University, Bangladesh.

(2) Mabel Aworh, Ahmadu Bello University, Nigeria.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/50368>

**Original Research Article**

**Received 12 May 2019**  
**Accepted 22 July 2019**  
**Published 05 August 2019**

### **ABSTRACT**

The study was designed to isolate predominance contamination of *Pseudomonas aeruginosa* in marketed raw buffalo milk (n=122) samples, collected from private dairy farms from different places of south Gujarat, India. Pre-enrichment of 1 ml of each sample was done with inoculation in 9 ml tryptone soya broth and incubated at 37°C for 24 hrs. A loopful of culture was taken from broth and streaked on selective *Pseudomonas* agar F plates and incubated at 37°C for 24 hrs, after completion of incubation period, the colonies characteristics were studied and further confirmed by various biochemical tests and found 14 samples contaminated with *P. aeruginosa*, were further more biochemical testes are used and give positive results with IMViC, Motility test, catalase and sugar fermentation confirm at 37°C for 24 hrs incubation. All biochemically conformed isolates

\*Corresponding author: Email: [rajeev37171@gmail.com](mailto:rajeev37171@gmail.com);

were further subjected for molecular characterization and were also tested for antimicrobial susceptibility by using various antibiotics discs such as vancomycin, penicillin, tylosin, cefixime, chloramphenicol, kanamycin, ceftriaxone, cefixime, tetracycline, streptomycin, ciprofloxacin, enrofloxacin, oxytetracycline and gentamicin, which has shown multi drug resistant ranging from seven to nine antimicrobials and Multiple Antibiotic Resistance (MAR) index ranges from 0.50 to 0.64. The isolates of *P. aeruginosa* in the present study are extremely resistant to vancomycin, penicillin, tylosin, cefixime, chloramphenicol and maximum sensitive to ciprofloxacin and enrofloxacin followed by gentamicin. Further statistical analysis of antibiotics wise zone diameter interpretative standard (mm) revealed susceptibility phenotypes under significant of difference at  $P \leq 0.05$  in one way ANOVA using Duncan's multiple range test and found ciprofloxacin having maximum sensitivity among antibiotic tested and it could be considered as a drug of choice for controlling *P. aeruginosa* mediated animal and human infections in the studied regions for insuring food safety as well.

**Keywords:** *Pseudomonas aeruginosa*; buffalo; milk; antimicrobial; resistant; therapeutics.

## 1. INTRODUCTION

The control of microbial spoilage of livestock originated products is crucial for the quality and safety of the foods [1] which requires an understanding of a number of factors including the knowledge and preventive management of possible hazards.

Raw and poor boiled milk is widely consumed in present times and its continuous increasing market demand with growing human population in the world. Among the foods of animal origin, milk is a significant food of human nutrition owing to its high nutritional value. It is naturally a good medium for growth of microorganisms. Raw milk and end products quality control are crucial areas for quality management in the dairy industry where biological contamination has an impact on food quality and safety, shelf life of consumer safety to reduce food poisoning outbreaks [2].

Milk is highly prone to contamination and can serve as an efficient vehicle for human transmission of foodborne pathogens, especially gram negative bacteria, as these are widely distributed in the environment [3]. Negligence of hygienic condition such as improper cleaning of bulk tank and milking equipments, dirty udders, milk handling technique and improper storage will increase and playing an important role in determining the quality of milk, which are one of the major sources of protein in a vegetarian's diet [4].

Among the spoilage bacteria, psychotropic bacteria have become an escalating problem in the dairy industry for several decades [5]. So, the psychrotrophs have received increased attention by investigators nowadays, because of modern

developments in the handling and transportation of milk for held for long period at refrigeration temperature before processing, manufacturing or consumption [6].

The main psychotropic bacteria present in raw milk are *Pseudomonas* species, comprising at half of the total bacteria in refrigerated foods where *P. aeruginosa* have been the critical cause in majority of outbreaks of various systemic infections like urinary, respiratory and gastrointestinal system, skin lesions, bone and joint infections etc. in whole family. Due to its nominal nutritional requirement *P. aeruginosa* has the ability to survive in environment [7].

*Pseudomonas* is gram negative motile aerobic rods and most heterogenous ecologically significant known bacteria which are important to contributing the milk spoilage were widely spread throughout nature. They are characterized by elevated metabolic activity because production of many thermo tolerant lipolytic and proteolytic complex enzymatic system [8].

*P. aeruginosa* is an opportunistic pathogen that can affect human, animal and birds and common in the farm environment particularly in supplies contaminated water used for udder washing and often observed as a source of infection for pseudomonas mastitis in cows. This organism is highly ubiquitous dangerous and dreaded pathogen in water system and capable of acquiring various antibiotic resistances due to its low outer membrane permeability and extensive efflux pump system. *P. aeruginosa* infection may cause economic loss due to its ability to reduce the quality of products due to infection transmitted through consumption of contaminated mastitis milk by

immunocompromised patients. However very small work was carried out on the isolation of *P. aeruginosa* from milk samples and the data is not proper uniform.

The global problem of antimicrobial resistance is particularly pressing in developing countries, where the infectious disease burden is high and cost constraints prevent the widespread application of newer, more expensive agents. Multiple antibiotic resistances (MAR) indexing has been shown to be a cost effective and valid method of bacteria tracking. It MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used [9,10]. High incidence, infection severity and increasing antibiotic resistance bacterium infections highlighting the need for new therapeutic and management option development [4].

The conventional microbiological methods are rapid, accurate and reliable for identifying spoilage bacteria as *P. aeruginosa* from raw milk samples through PCR is very important in the efficient monitoring of microbiological qualities, especially in raw and ready to eat foods [11]. Molecular approaches, especially those based on the use of 16S rRNA genes (DNA) and related techniques, have provided the opportunity to analyze complex communities on the basis of sequence diversity [12,10].

Keeping in view of the severity of the infections caused by *P. aeruginosa* among the animal population and its public health significance this study was carried out to:

- 1) Isolation and identification of *P. aeruginosa* from milk using bacteriological and biochemical methods.
- 2) Determination of the antibiotic resistant pattern and MAR index of the isolates with therapeutics antibiotic.
- 3) Application of conventional PCR depending on 16S rDNA sequences on the isolates to identify genus of *Pseudomonas*.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

A total of 122 marketed buffalo milk samples were collected during Sept. 2017 to Feb. 2018 for isolation and identification of *P. aeruginosa* from non organized private dairy farms in and overall Navsari and Surat district of south

Gujarat, India. Milk sample of 20 ml was collected aseptically packed in 3 x 4 cm sterile autoclaved milk collection falcon tube from each animal with proper labeling, such as date, place, species etc. Immediately after collection, all the samples were kept in thermo flask maintaining temperature about 4°C - 8°C and transported to the laboratory for further processing.

### 2.2 Microbiological Analysis

#### 2.2.1 Isolation and identification of *P. aeruginosa*

Before processing, the milk samples were thoroughly mixed aseptically and from each collection tube 1 ml milk was drawn with the help of sterile pipette and transferred into 10% marked 9 ml enrichment Tryptone Soya Broth (Himedia Pvt. Ltd, India) in separate test tube and incubated at 37°C for 24 hrs. After incubation, a loopful of culture was taken and streaked on Pseudomonas agar (for fluorescein) medium (Himedia Pvt. Ltd, India) and plates were incubated at 37°C for 24 hrs. Pseudomonas agar (for fluorescein) is a special medium for the identification of *P. aeruginosa* enhances the elaboration of fluorescein by *Pseudomonas* and inhibits the pyocyanin coloration.

#### 2.2.2 Biochemical characterization

The suspected colonies on Pseudomonas agar (for fluorescein) was collected and screened for gram staining and biochemical characterization which includes indole, methyl red, voges proskauer, citrate utilization, triple sugar iron, lysine iron agar, mannitol motility, gelatin hydrolysis, oxidase, catalase and sugar fermentation tests. All these tests were performed according to the protocols as mentioned [13].

#### 2.2.3 Determination of antibiotic resistance pattern

The isolated conformed *P. aeruginosa* was challenged for 13 different antibiotic used in therapeutics medicine was selected and resistance pattern was obtained as per guidelines from CLSI [14,15] using disc diffusion method (D-test) on Mueller Hinton agar using commercial discs (Himedia Pvt. Ltd, India). Initially the test was performed by inoculating the test colonies in Mueller Hinton broth and incubated at 37°C for 6 - 8 hrs. Later on, Muller Hinton agar plates were swabbed with Muller Hinton broth culture with the

help of sterile swab and antibiotic discs were placed carefully on swabbed surface of plates were incubated at 37°C for 24 hrs and zones of inhibition for different antibiotics were measured and result was computed.

### 2.3 MAR Index

The multiple antibiotic resistance indexes (MAR index) were determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested [16] and results were presented.

MAR index = Number of antibiotics to which isolate is resistant / Total number of antibiotics used in this test

### 2.4 Molecular Characterization of *P. aeruginosa*

The *Pseudomonas* species isolated from marketed raw buffalo milk samples were confirmed by targeting 16S rDNA gene of *Pseudomonas* species using PCR.

### 2.5 DNA Extraction

The isolated colonies were then cultured in Luria-Bertani broth and incubated at 37°C for 24 hrs. DNA extraction from samples was performed using the *mericon* DNA Bacteria plus Kit (Qiagen, Germany) with some modifications from the manufacturer's recommendations. Following the incubation, 2 ml bacterial culture was centrifuged at 12000 rpm for about 10 min. Then add with 200µl NFW for washing and again centrifuged at 10000 rpm for about 5 min. To the pellet 200 µl of lysis buffer was added and vortexed properly and incubated at 100°C in heating block for 10-15 min. After cool at room temperature and centrifuged at 12000 rpm for about 10 min. To the upper aqueous layer was collected in new centrifuge tube and store at -20°C for 2 - 3 months. The extracted DNA was then quantified using the NanoDrop™2000/2000c spectrophotometer to check for the purity.

### 2.6 Oligonucleotide Primers

The sequence of first set of primers F - GACGGGTGAGTAATGCCTA and R - CACTGGTGTTCTTCCTATA obtained from published work [17] was specific for 16S rDNA gene of the genus *Pseudomonas* species were

commercially synthesized from Eurofins Genomic, India.

### 2.7 PCR Amplification of DNA Samples

Primers were utilized in a 25 µl reaction containing 12.5 µl of 2X PCR Master Mix (Qiagen, Germany), 1 µl of each primer of 20 pmol concentrations, 5.5 µl of NFW and 6 µl of DNA template. The reaction was performed in Thermal cycler (Bio-Rad S1000™ Thermal cycler, Sweden).

### 2.8 Analysis of the Conventional PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel stain with Ethidium Bromide (Appllichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 10 µl of the products was loaded in each gel slot. A 100 bp plus DNA Ladders (Qiagen, Germany) were used to determine the fragment sizes. The gel was photographed by a Gel documentation system (Bio-Rad Gel Doc™ XR+Gel Documentation system, Sweden) and the data was analyzed through computer software.

### 2.9 Statistics Analysis

The antibacterial resistance and susceptibility data were expressed as means ± standard deviations (SD) and compared using one-way ANOVA in SPSS software (version 22.0 for Windows, SPSS Inc.) was explained in terms of percentage and recorded in tabular form, while to estimate resistance pattern of antibiotics wise zone diameter interpretative standard (mm) revealed susceptibility phenotypes under significant of difference at  $P \leq 0.05$  in one way ANOVA using Duncan's multiple range test.

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and Biochemical Characterization of *Pseudomonas* Species

In this study 14 isolates of *Pseudomonas* species was isolated from 122 marketed raw buffalo milk samples in a percentage of (11.48%). The colonies were cultured on *Pseudomonas* Agar plates and colonies appeared fluorescent yellow or green color was subject for further confirmation by Gram's Stain. The isolated

*Pseudomonas* strains were biochemically characterized by IMViC. All 14 isolates, showed slant / butt – acidic / acidic or yellow / yellow, H<sub>2</sub>S and gas - negative on Triple Sugar Iron (TSI) and Lysine Iron Agar showed slant/ butt - alkaline/ acidic or purple /yellow, H<sub>2</sub>S and gas negative. All the isolates also showed positive reaction for citrate utilization, oxidase, catalase, motility, gelatin hydrolysis and ferment only D-mannitol but did not ferment D-maltose, D-xylose, and Ethylene glycol in sugar fermentation test. All the isolates were found negative to indole, Methyl red and voges proskauer reaction and pure isolates were identified as *P. aeruginosa* contamination in relation to source or origin.

Microbial analysis of raw milk has a critical role to evaluate its quality and promoting public health safety. Recently, food borne illnesses outbreaks were associated with raw milk consumption, found to be contaminated with pathogenic microorganisms such as *Pseudomonas spp.* [18]. The present work was made in order to evaluate the prevalence of *Pseudomonas* species among raw milk using conventional PCR.

A total of 122 samples of raw milk were examined for presence of *Pseudomonas* species contamination. The percentage of *Pseudomonas* species was 11.48 % (14 isolates). All the 14 isolates by biochemical identification were identified as *P. aeruginosa*. These results were at least higher to those reported in [19], who isolated *P. aeruginosa* from raw milk samples in proportion of 6.6%. However, the current results were similar to those recorded in [20], they showed that *P. aeruginosa* was isolated from 9.5% and 11.11%. The difference between our results and the previous studies may be attributed to sampling techniques, sources of sampling, handling of samples and types of media. The presence of *P. aeruginosa* in the milk samples were due to contamination and unhygienic maintenance polluted water at farm level.

### 3.2 Determination of Antibiotic Resistance Pattern

The Muller Hinton agar based antibiogram resistance and sensitivity pattern study of *P. aeruginosa* isolated from milk is represented diagrammatically in Fig. 1. The isolates of *P. aeruginosa* in the present study are extremely resistant to vancomycin, penicillin, tylosin, cefixime and chloramphenicol maximum

sensitive to ciprofloxacin and enrofloxacin followed by gentamicin. Antidrug profile of Mean±SE values of antibiotics wise zone diameter of *P. aeruginosa* is represented in Table 1.

Along with penicillin was also shown 100 % resistance to the isolates of *P. aeruginosa* in the present investigation. These results are in accordance with the results mention in [21,22], who also reported 100% resistance to penicillin by the isolates of *P. aeruginosa*.

The isolates of *P. aeruginosa* in the present investigation have shown 64.29% intermediate resistance to tetracycline. Similar to these results [23,24,25] have reported 75, 81 and 83.3% resistance to tetracyclines by their isolates of *P. aeruginosa* respectively.

The isolates of *P. aeruginosa* in the present investigation have shown 92.85% intermediate resistance to ceftriaxone. Similarly to the results obtained in the study [21,24,26] have reported 65%, 45.4% and 100% sensitive to *P. aeruginosa* respectively.

In the present study, ciprofloxacin has shown 100% of sensitive by the isolates of *P. aeruginosa*. Similarly to the results obtained in the study, [16,22,23,27] have reported 57%, 73%, 100% and 69.8% sensitive to *P. aeruginosa* respectively. *P. aeruginosa* has shown a bit more resistance to ciprofloxacin in the works carried out by [28,29,30] who reported 92, 60 and 56% respectively.

Gentamicin and ciprofloxacin are considered potent agents in the treatment of infections caused by multi-resistant *P. aeruginosa*. Gentamicin has shown 100 % sensitive to the isolates of *P. aeruginosa* in the present investigation. Similarly to the results obtained in the study [23,31,32,33] have reported 100%, 88.50%, 88% and 75% sensitive to *P. aeruginosa* respectively. In contrast study [25,26,27], who reported 66.6%, 85% and 100% of resistance to gentamicin by *P. aeruginosa* their isolates respectively.

Chloramphenicol has shown 100% resistance to *P. aeruginosa* isolated in this study. Similar to the results of present investigation [23] reported 63.9 % of resistance by their isolates where as [26,27,30] have reported 100%, 98.1% and 73.7% resistance to chloramphenicol by the isolates of *P. aeruginosa*.

This high resistance may be due to the fact that isolated strains from milk samples have been subjected to the selective action of both antibiotics and disinfectants. The isolates have shown cent percent resistance to vancomycin, penicillin, tylosin, cefixime and chloramphenicol where as maximum sensitive to ciprofloxacin and gentamicin (100%) followed by enrofloxacin and streptomycin (92.85%). In this study, the isolates shown resistance to ceftriaxone (92.85%), oxytetracycline (85.71%) and kanamycin (78.57%). Tetracycline has shown 64.29 % intermediate resistance for *P. aeruginosa* isolates in this study. These results are indicating that there is an emergence of multidrug resistant *P. aeruginosa*.

### 3.3 MAR Index

These results are indicating that there is an emergence of multidrug resistant *P. aeruginosa* given in the Table 2. All the isolates of *P. aeruginosa* have shown multi drug resistant ranging from seven to nine antibiotics which is almost similar to studies reported in [34]. All the 14 isolates of *P. aeruginosa* in the present study have shown different MAR indices ranging from 0.50 to 0.64. Because of mutations in outer membrane pores resulting in reduced permeability to antimicrobials and also due to over expression of multi drug efflux pumps, which tends to pump out antibiotics before they have opportunity to act on their target results in

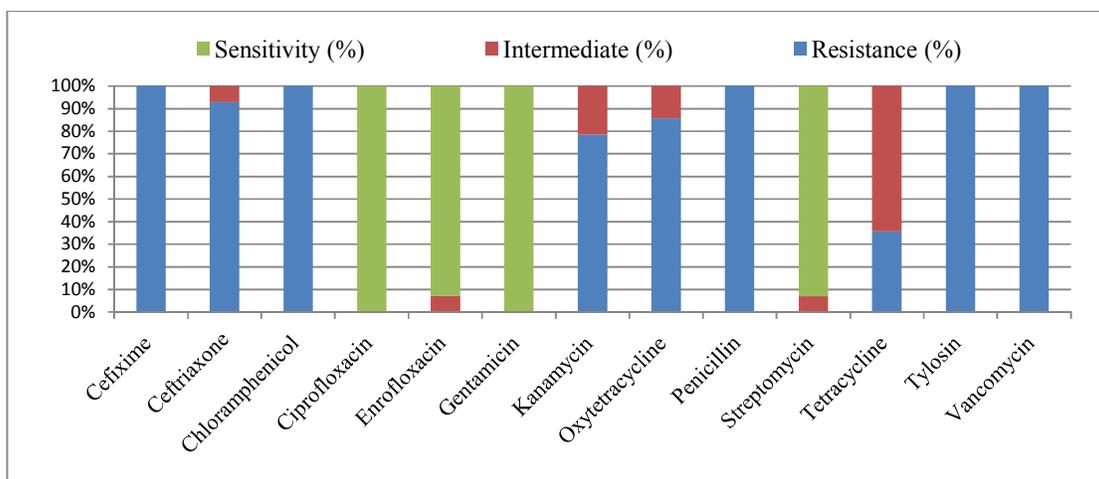


Fig. 1. Antimicrobial resistance and sensitivity pattern of *P. aeruginosa* isolates

Table 1. Mean±SE values of antibiotics wise zone diameter interpretative standard of *P. aeruginosa*

Sr. no.	Antibiotics wise zone diameter interpretative standard (mm)	
	Antibiotic	Mean±SE
1	Cefixime	10.43 <sup>a</sup> ±0.31
2	Ceftriaxone	10.00 <sup>a</sup> ±0.00
3	Chloramphenicol	27.29 <sup>f</sup> ±0.83
4	Ciprofloxacin	16.21 <sup>c</sup> ±0.45
5	Enrofloxacin	25.93 <sup>e</sup> ±0.65
6	Gentamicin	21.21 <sup>d</sup> ±0.52
7	Kanamycin	12.29 <sup>b</sup> ±0.29
8	Oxytetracycline	11.29 <sup>ab</sup> ±0.40
9	Penicillin	10.00 <sup>a</sup> ±0.00
10	Streptomycin	16.57 <sup>c</sup> ±0.56
11	Tetracycline	11.00 <sup>a</sup> ±0.41
12	Tylosin	10.14 <sup>a</sup> ±0.14
13	Vancomycin	10.00 <sup>a</sup> ±0.00

Mean bearing different superscripts (<sup>a,b,c,d,e & f</sup>) in column differ significantly at  $p \leq 0.05$ , One way ANOVA using Duncan's multiple range test, analysis with SPSS software (version 22.0 for Windows, SPSS Inc.)



**Fig. 2. Agarose gel electrophoresis PCR amplified product of 618 bp for 16S rRNA gene of *Pseudomonas* spp. isolates, MWM - molecular weight marker (100 bp plus DNA ladder), + control (Positive, Negative) + different strains of *Pseudomonas* spp.**

**Table 2. MAR Index of *P. aeruginosa* isolated from marketed buffalo raw milk**

Sample no.	MAR index	Antibiotics
1	0.50	VA, P, K, TL, CFM, C, OTC
2	0.57	VA, P, K, CTR, TL, CFM, C, OTC
3	0.64	VA, P, K, CTR, TE, TL, CFM, C, OTC
4	0.57	VA, P, K, CTR, TL, CFM, C, OTC
5	0.64	VA, P, K, CTR, TE, TL, CFM, C, OTC
6	0.57	VA, P, CTR, TE, TL, CFM, C, OTC
7	0.64	VA, P, K, CTR, TE, TL, CFM, C, OTC
8	0.64	VA, P, K, TL, CTR, TE, CFM, C, OTC
9	0.57	VA, P, CTR, TE, TL, CFM, C, OTC
10	0.64	VA, P, K, CTR, TE, TL, CFM, C, OTC
11	0.57	VA, P, K, CTR, S, TL, CFM, C, OTC
12	0.64	VA, P, K, CTR, TE, TL, CFM, C, OTC
13	0.64	VA, P, K, CTR, TE, TL, CFM, C, OTC
14	0.64	VA, P, K, CTR, TE, TL, CFM, C, OTC

Note: VA - Vancomycin, P - Penicillin G, K - Kanamycin, CTR - Ceftriaxone, CFM - Cefixime, TE - Tetracycline, TL - Tylosin, OTC - Oxytetracycline, S - Streptomycin

bacterial resistance to multi drug antibiotics [34]. The multiple resistance development of therapeutics antimicrobial resulting in to challenging means of insuring food safety for consumers as observed in this study.

### 3.4 Molecular Characterizations of *P. aeruginosa*

In our study *P. aeruginosa* was confirmed by targeting 16S rDNA gene of *Pseudomonas* species by conventional PCR and successfully amplified the desired amplicon product. The results proved that the isolates were *P. aeruginosa* as mention in Fig. 2. All 14 isolates were identified in laboratories as species other than *Pseudomonas* species similar to the results also shown in [35], who reported 6 isolates confirmed by this primer pair.

## 4. CONCLUSION

This study has shown that the rate of antibiotic resistance pattern against *P. aeruginosa* is medium at studied region and bacterium

contamination is a prudent and justifiable reasons for un judicial antibiotics consumption both for prophylactic and therapeutic against mastitis and other infections should be critically evaluated against the effects of antimicrobial resistance. Effective management of MDR *P. aeruginosa* in the milk for insuring food safety for consumers by veterinarians, would require good background knowledge of the prevailing antimicrobial susceptibility pattern of the organism. Such information would be even more valuable in human and animals health care medicine of the country, where reports about the occurrence of antimicrobial resistant *P. aeruginosa* are increasing day by day especially with urinary tract infections is a challenging means of insuring food safety and therapeutic strategies.

## ACKNOWLEDGEMENT

The authors are grateful to the Dean, College of Veterinary science & Animal Husbandry and Director of Research, Navsari Agricultural University. Navsari-396 450, Gujarat, India for

providing necessary facility and resources to conduct this study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

- Zhang SB, Zhai HC, Hu YS, Wang L, Yu GH, Huang SX, Cai JP. A rapid detection method for microbial spoilage of agro-products based on catalase activity. *Food Control*. 2014;42:220-224.
- Bashir S, Awan MS, Khan SA, Suthar V, Khan MM, Khan MT. Microbiological quality evaluation of raw milk consumed in and around Rawalakot, Azad Jammu and Kashmir. *International Journal of Microbiology Research*. 2014;5:112-116.
- Garedew L, Berhanu A, Mengesha D, Tesgay G. Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. *BMC Public Health*. 2012;12:950. Available:<http://www.biomedcentral.com/1471-2458/12/950>.
- Kumar R, Singh SP, Kumar M, Kumar A. Determination of low molecular weight immunogenic Omp28 Protein and Gene of *Salmonella typhimurium*. *South Asian Journal of Experimental Biology*. 2018;8(5):172-177.
- Singh P, Wani AA, Karim AA, Langowski AA. The use of carbon dioxide in the processing and packaging of milk and dairy products: A review. *International Journal of Dairy Technology*. 2012;65:161-177.
- Oifa SB, Imène F, Rouaa L, Hamadi A, Mohamed AA. Study of proteolytic and lipolytic Activities of *Pseudomonas* spp. Isolated from pasteurized milk in Tunisia. *Journal of Agricultural Science*. 2013;5(7):46-50.
- Aylin A, Evrim GA, Gokce PY. A modified selective medium containing Benzalkonium Chloride (BKC) for the isolation of *Pseudomonas aeruginosa* from raw milk. *Food and Nutrition Sciences*. 2012;3(7):947-950.
- Wiedmann M, Weilmeier D, Dineen SS, Ralyea RM, Boor KJ. Molecular and phenotypic characterization of *Pseudomonas* spp. isolated from milk. *Applied Environment Microbiology*. 2000;66(5):2085-2095.
- Osundiya OO, Oladele RO, Oduyebo OO. Multiple Antibiotic Resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. *African Journal of Clinical and Experimental Microbiology*. 2013;14(3):164 -168.
- Kumar R, Dutta S, Pal D. Microbiological quality assessment and detection of fecal *Escherichia coli* (Coliform) in foods of animal origin sold in the open markets of Kolkata city in India. *Indian Journal of Comparative Microbiology Immunology and Infectious. Diseases*. 2017;38(1):22-25.
- Arakawa K, Kawai Y, Iioka H, Tanioka M, Nishimura J, Kitazawa H, Tsurumi K, Saito T. Microbial community analysis of food-spoilage bacteria in commercial custard creams using culture-dependent and independent methods. *Journal of Dairy Science*. 2008;91(8):2938-2946.
- Kumar R, Singh SP. Pulse field gel electrophoresis-profiles of *salmonella* enteric serovar *typhimurium* isolates obtained from chickens sources. *Indian Journal of Comparative Microbiology Immunology and Infectious, Diseases*. 2014;35(20): 64-66.
- Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB. [1984 (Williams & Wilkins)]. George M. Garrity (ed.). the firmicutes. *Bergey's manual of systematic bacteriology*. 3 (2<sup>nd</sup> ed.). New York: Springer. 2009;1450. ISBN 978-0-387-95041-9. British Library no. GBA561951.
- CLSI. Performance standards for antimicrobial susceptibility testing., 27<sup>th</sup> ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA 19087 USA; 2017.
- Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*. 1966;45(4):4593-4596.
- Piyush Tripathy, Gopa Banerjee, Shivani Saxena, Mahendra Kumar Gupta, Ramteke PW. Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from patients of lower respiratory tract infection.

- African Journal of Microbiology Research. 2011;5(9):2955-2959.
17. Spilker T, Coenye T, Vandamme P, Li Puma JJ. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. *Journal of Clinical Microbiology*. 2004;42(5):2074-2079.
  18. Nawaz MA, Bhattarai RR. Isolation and identification of *Yersinia* and *Pseudomonas* spp. from Australian milk and salad using 16S rDNA. *Peer J. Pre Prints*. 2015;3:e820v1. Available:<https://dx.doi.org/10.7287/peerj.p.reprints.820v1>.
  19. El-Zubeir IEM, El-Owni OAO. Antimicrobial resistance of bacteria associated with raw milk contaminated by chemical preservatives. *World Journal of Dairy & Food Sciences*. 2009;4 (1):65-69.
  20. Jyoti Y, Saurav P, Jyotsna KP, Yashab K, Ajay K, Singh Florin M, Harison M. Comparative evaluation of pathogenic bacterial incidence in raw and pasteurized milk. *International Journal of Engineering Science Invention*. 2014;3(5):11-20.
  21. Jombo GTA, Jonah P, Ayeni JA. Multidrug resistant *Pseudomonas aeruginosa* in contemporary medical practice: Findings from urinary isolates at a Nigerian University Teaching Hospital. *Nigerian Journal of Physiological Sciences*. 2008;23(1-2):105-109.
  22. Hajira Bilal, Fariha Hasan, Samina Bilal. Susceptibility pattern of *Pseudomonas aeruginosa* against various antibiotics along with computational analysis. *International Journal of Sciences: Basic and Applied Research*. 2015;24(4):23-45.
  23. Gedamu Shewatatek, Gizachew Tilahun, Molalegne Bitew, Terefe Gelibo. Drug sensitivity of *Pseudomonas aeruginosa* from wound infections in Jimma University Specialized Hospital, Ethiopia. *Online Journal of Medicine and Medicinal Research*. 2014;3(2):13-18.
  24. Garba I, Lusa H, Bawa E, Tijjani MB, Aliyu MS, Zango UU, Raji MIO. Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolated from wounds in patients attending ahmadu bello university teaching hospital, Zaira, Nigeria. *Nigerian Journal of Basic and Applied Science*. 2012;20(1):32-34.
  25. Tamil SS, Murugan S. Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* from diabetes patients with foot ulcers. *International Journal of Microbiology*. 2011;4. Article ID 605195 Available:<https://doi.org/10.1155/2011/605195>.
  26. Gad GF, Ramadan A, El-Domany, SZ, Hossam MA. Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: Prevalence, antibiogram and resistance mechanisms. *Journal of Antimicrobial Chemotherapy*. 2007;60: 1010-1017.
  27. Okon KO, Aguwe PC, Oladosu W, Balogun Uba A. Antibiotic resistance patterns of *Pseudomonas aeruginosa* isolated from clinical specimens in a tertiary care hospital in North eastern Nigeria. *Journal of Microbiology and Antimicrobials*. 2009;1(2):19-26.
  28. Al-Kabsi AM, Yusof M, Sekaran SH. Antimicrobial resistance pattern of clinical isolate of *Pseudomonas aeruginosa* in the University of Malaya Medical Center, Malaysia. *African Journal of Microbiology*. 2011;5:5266-5272.
  29. Zaheer Ali, Nusrat M, Sehar ANNJ, Maryam S. Multi-drug resistant *Pseudomonas aeruginosa*: A threat of nosocomial infections in tertiary care hospitals. *Journal of the Pakistan Medical Association*. 2015;65(1):12-16.
  30. Ahmed BM, Ahmed ZW, Rashad HG, Zaghlol LA, Galal R. Prevalence of Multi drug resistant *Pseudomonas aeruginosa* in patients with nosocomial infection at a University Hospital in Egypt, with special reference to typing methods. *Journal of Virology and Microbiology*. 2013;01-13.
  31. Ahmad BM, Kudi MT. Chronic suppurative otitis media in Gombe, Nigeria. *Nigerian Journal of Surgical Research*. 2003;5(3):120-123.
  32. Ferede D, Geyid A, Lulseged S, Melaku A. Drug susceptibility pattern of bacterial isolates from children with chronic suppurative otitis media. *Ethiopian Journal of Health Development*. 2001;15(2):89-96.
  33. Ogundipeju OO, Nwobu RAU. Occurrence of *Pseudomonas aeruginosa* in post-operative wound infection. *Pakistan Journal of Medical*. 2004;20:187-191.
  34. Emmanuel EO, Etinosa OI, Raphael M, Bright I, Clara LI, Anthony IO. Prevalence of Multiple Antibiotic Resistant (MAR)

- Pseudomonas* species in the final effluents of three municipal waste water treatment facilities in south Africa. International Journal of Environmental Research and Public Health. 2012;9(6):2092-2107.
35. Mohammed GMO, Megahed AA, Nasr SS. Bacteriological and molecular detection of pseudomonas species from raw milk sold in Port-Said City markets. Egyptian Journal of Chemistry and Environmental Health. 2015;1(1):986-1002.

---

© 2019 Patel et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle3.com/review-history/50368>