Original Research Article

Resistant Patterns of Therapeutics Antimicrobial challenged on *Pseudomonas Aeruginosa* Bacterium Isolated from Marketed Raw Buffalo Milk

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^{o}C for 24 hrs. A loopful of culture was taken from broth and streaked on selective Pseudomonas agar F plates and incubated at 37^{o}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^{o}C for 24 hrs incubation. All biochemically conformed isolates 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) reveled 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 (Zhang *et al.,* 2014) which requires an understanding of a number of factors including the knowledge and preventive management of possible hazards , their likely occurrence in different products, their physiological properties and the availability and effectiveness of different preventive practices.

Raw and poor boiled milk is widely consumed in present times and its market demand is continuous increase due to increasing 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 in process and end product 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 and consumer safety of dairy products (Blackburn, 2006). Quality control raw milk is therefore of paramount importance with a view to reduce food poisoning outbreaks (Bashir *et al.,* 2014).

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 (Garedew *et al.,* 2012). Negligence of hygienic condition such as improper cleaning of bulk tank, dirty udders, milking equipments, milk handling technique and improper storage will increase the proportion of gram negative bacteria in the bulk tank milk (Bonfoh *et al.,* 2003; Kumar *et al.,* 2018). These are playing an important role in determining the quality of milk, which are one of the major sources of protein in a vegetarian's diet.

Among the spoilage bacteria, psychotropic bacteria have become an escalating problem in the refrigerated storage and distribution of fluid milk and dairy products for the food industry for several decades (Singh *et al.,* 2012). 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 (Olfa *et al.,* 2013).

The main psychotropic bacteria present in raw milk are gram negative rods i.e. *Pseudomonas* species, comprising at half of the total bacteria in refrigerated foods (Champagne *et al.,* 1994) where *P. aeruginosa* have been the critical cause in majority of outbreaks of inflammatory infections in whole family. It is mainly responsible for a variety of systemic infections like urinary, respiratory and gastrointestinal system, skin lesions, bone and joint infections etc. Due to its nominal nutritional requirement *P. aeruginosa* has the ability to survive in soil, plant surface, waste water, moist environment, surface waters or even on inert materials (Aysel *et al.,* 2012).

Pseudomonas is gram negative motile aerobic rods and most heterogonous 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 (Widemann *et al.,* 2000). Although *Pseudomonas* bacteria are easily killed from contaminates

processed milk by pasteurization therefore *Pseudomonas* species are the most important bacteria responsible for spoilage of pasteurized milk during refrigerated storage (Smithwell and Kailasapathy, 1995).

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. 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 isolate *P. aeruginosa* from milk and to determine the antibiotic resistant patterns of the isolates to some commonly used antibiotics. High incidence, infection severity, and increasing antibiotic resistance characterize *P. aeruginosa* infections highlighting the need for new therapeutic and management options (Kipnise *et al.,* 2006; Kumar *et al.,* 2018).

The rapid, accurate and reliable identification of spoilage bacteria through PCR is very important in the efficient monitoring of microbiological qualities, especially in raw and ready to eat foods (Arakawa *et al.,* 2008). Molecular approaches, especially those based on the use of 16S rRNA genes (DNA) and related techniques, have provided the opportunity to analyse complex communities on the basis of sequence diversity. Bacterial species can be identified by generating clone libraries of the *16S rDNA* followed by sequencing and comparison with databases containing thousands of ribosomal sequences to allow a phylogenetic affiliation to cultured, as well as uncultured microorganisms (Ercolini, 2004). Conventional microbiological methods for identifying *P. aeruginosa* from raw milk samples are reliable and require several days to be completed. PCR has the potential for identifying microbial species rapidly and specific by amplification of sequences unique to a particular organism (Khan and Cerniglia, 1994).

2. Materials and Methods

1) Sample collection and preparation

A total of 122 marketed buffalo milk samples was collected 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^{o}C - 8^{o}C and transported to the laboratory for further processing.

2) Microbiological analysis:

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^{O} 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^{O} 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) Biochemical characterization

The suspected colonies on Pseudomonas agar (for fluorescein) was collected and screened for grams 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 mentioned *Burgey's Manual of Systemic Bacteriology,* 2009.

3) Determination of antibiotic resistance patterns

The isolated conformed *P. aeruginosa* was challenged for 13 different antibiotic used in therapeutics medicine was selected and resistance patters was obtained as per guidelines from CLSI (2017) and Kirby Bauer (1966) 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^{O} 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^{O} C for 24 hrs and zones of inhibition for different antibiotics were measured and result was computed.

3.1) MAR index

The multiple antibiotic resistance index (MAR index) was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (Piyush Tripathy *et al.,* 2011) and results were presented.

$$\text{MAR index} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics used in this test}}$$

4) Molecular characterization of *P. aeruginosa*

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

4.1) 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, 2ml 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 minutes. To the pellet 200 µl of lysis buffer was added and vortexed properly and incubated at 100^{o}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^{0}C for 2 - 3 months. The extracted DNA was then quantified using the NanoDrop™2000/2000c spectrophotometer to check for the purity

4.2) Oligonucleotide primers

The sequence of first set of primers *F*- GACGGGTGAGTAATGCCTA and R – CACTGGTGTTCCTTCCTATA obtained from published work (Spilkar *et al.,* 2004) was specific for *16S rDNA* gene of the genus Pseudomonas species were commercially synthesized from Eurofins Genomic, India.

4.3) 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 S1000TM Thermal cycler, Sweden).

4.4) Analysis of the Conventional PCR products

The products of PCR were separated by electrophoresis on 1.5% agarose gel stain with Ethidium Bromide (Applichem, 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^{TM} XR+Gel Documentation system, Sweden) and the data was analyzed through computer software.

5) **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 patterns of antibiotics wise zone diameter interpretative standard (mm) reveled susceptibility phenotypes under significant of difference at $P \leq 0.05$ in one way ANOVA using Duncan's multiple range test.

RESULTS AND DISCUSSION

1) Isolation and biochemical characterization of *Pseudomonas* species

In this study 14 isolates of *Pseudomonas* species was isolated from 122 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 were 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_2S and gas - negative on Triple Sugar Iron (TSI) and Lysine Iron Agar showed slant/ butt - alkaline/ acidic or purple /yellow, H_2S and gas negative. All the isolates also showed positive reaction for citrate utilization, oxidase, catalse, 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, promoting public health safety (Nicolaon and Goodacre, 2008). Recently, food borne illnesses outbreaks were associated with raw milk consumption, found to be contaminated with pathogenic microorganisms such as *Pseudomonas spp.* (Nawaz and Bhattarai, 2015).

Milk contaminated with disease causing bacteria does not smell or look any differ from normal milk, and there is no obvious way for the consumer to know if the milk is contaminated (Julia, 2010).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. aerugenosa*. These results were at least higher to those reported by El- Zubeir and El- Owni (2009) who isolated *P. aerugenosa* from raw milk samples in proportion of 6.6%. However, the current results were similar to those recorded by Jyoti *et al.* (2014) and Aylin *et al.* (2012) they showed that *P. aerugenosa* was isolated from 9.5% and11.11% and higher than those found by Hussein (2008) who isolated *P. aerugenosa* in a percentage of 3.7% from raw milk samples. 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.

2) Determination of antibiotic resistance patterns

The Muller Hinton agar based antibiogram resistance and sensitivity pattern study of *P. aeruginosa* isolated from milk is shown in Table 1 and 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. aeroginosa* is represented in Table 2.

Table 1: Antimicrobial resistance and sensitivity pattern of *P. aeruginosa*

Sr. No.	Antibiotics drugs	Resistance (%)	Intermediate (%)	Sensitivity (%)
1	Cefixime (5 mcg)	100.00	0.00	0.00
2	Ceftriaxone (30 mcg)	92.85	7.15	0.00
3	Chloramphenicol (30 mcg)	100.00	0.00	0.00
4	Ciprofloxacin (5 mcg)	0.00	0.00	100.00

5	Enrofloxacin (10 mcg)	0.00	7.15	92.85
6	Gentamicin (10 mcg)	0.00	0.00	100.00
7	Kanamycin (30 mcg)	78.57	21.43	0.00
8	Oxytetracycline (30 mcg)	85.71	14.29	0.00
9	Penicillin (10 units)	100.00	0.00	0.00
10	Streptomycin (10 mcg)	0.00	7.14	92.85
11	Tetracycline (30 mcg)	35.71	64.29	0.00
12	Tylosin (15 mcg)	100.00	0.00	0.00
13	Vancomycin (30 mcg)	100.00	0.00	0.00

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 of Jombo *et al.* (2008) and Hajira *et al.* (2015) 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 Gedamu *et al.* (2014), Garba *et al.* (2012) and Tamil *et al.* (2011) have reported 75, 81 and 83.3% resistance to tetracyclines by their isolates of *P. aeruginosa* respectively. Whereas as very little percentage (10%) of resistance to tetracyclines by *P. aeruginosa* isolates was reported by Ogundipeju and Nwobu (2004).

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, Ged *et al.* (2007), Jombo *et al.* (2015), Deepak *et al.* (2011) and Garba *et al.* (2012) have reported 100 %, 65 %, 52 % and 45.4 % 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, Gedmu *et al.* (2014), Hajira *et al.* (2015), Okan *et al.* (2009) and Tripathi *et al.* (2011) have reported 100 %, 73 %, 69.8 % and 57 % sensitive to *P. aeruginosa* respectively. *P. aeruginosa* has shown a bit more resistance to ciprofloxacin in the works carried out by Alkabsi *et al.* (2011), Zaheer *et al.* (2012) and Ahmed *et al.* (2013) 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, Gedmu *et al.* (2014), Ahmad *et al.* (2004), Ferede *et al.* (2001) and Ogundipeju and Nwobu (2004) have

reported 100 %, 88.50 %, 88 % and 75 % sensitive to *P. aeruginosa* respectively. In contrast study, Okon *et al.* (2009), Gad *et al.* (2007) and Tamil *et al.* (2011) who reported 100%, 85% and 66.6% 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 Gedamu *et al.* (2014) reported 63.9% of resistance by their isolates where as Gad *et al.* (2007), Okon *et al.* (2009), Ahmed *et al.* (2013) 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*.

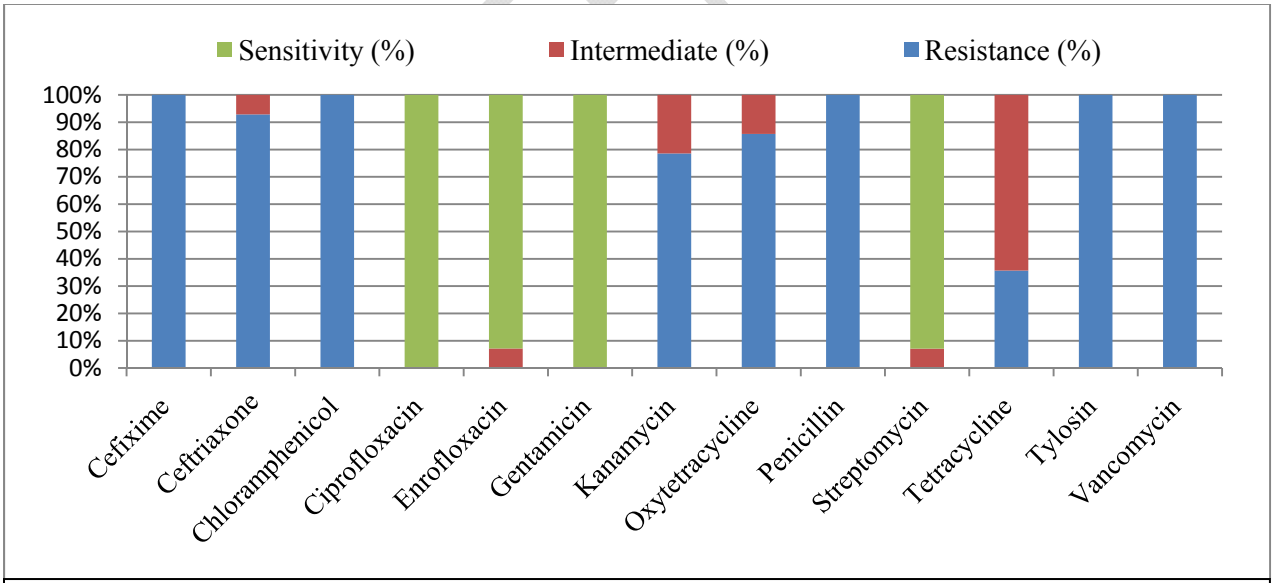

Fig. 1 Antimicrobial resistance and sensitivity pattern of *P. aeruginosa* isolates

Table 2: 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^{a}±0.31
2	Ceftriaxone	10.00^{a}±0.00
3	Chloramphenicol	27.29^{f}±0.83
4	Ciprofloxacin	16.21^{c}±0.45
5	Enrofloxacin	25.93^{e}±0.65
6	Gentamicin	21.21^{d}±0.52
7	Kanamycin	12.29^{b}±0.29
8	Oxytetracycline	11.29^{ab}±0.40
9	Penicillin	10.00^{a}±0.00
10	Streptomycin	16.57^{c}±0.56
11	Tetracycline	11.00^{a}±0.41
12	Tylosin	10.14^{a}±0.14
13	Vancomycin	10.00^{a}±0.00
Mean bearing different superscripts ($^{a,b,c,d,e\ \&\ f}$) in column differ significantly at ***P*≤0.05,** One way ANOVA using Duncan's multiple range test, analysis with SPSS software (version 22.0 for Windows, SPSS Inc.)		

3) MAR Index

These results are indicating that there is an emergence of multidrug resistant *P. aeruginosa* given in the Table 3. All the isolates of *P. aeruginosa* have shown multi drug resistant ranging from seven to nine antibiotics which is almost similar to studies of Lateef (2004) and Emmanuel *et al.* (2012). 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 bacterial resistance to multi drug antibiotics (Emmanuel *et al.,* 2012). The multiple resistance development of therapeutics antimicrobial resulting in to challenging means of insuring food safety for consumers as observed in this study.

Table 3: 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

4) Molecular characterizations of *P. aeruginosa*

I 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 Amal *et al.* (2015) who reported 6 isolates confirmed by this primer.

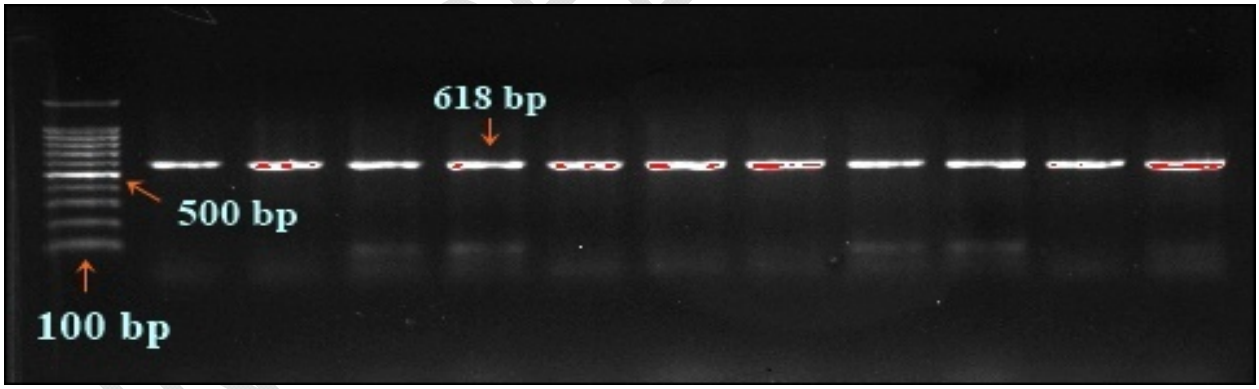

Fig. 3: Agarose gel electrophoresis PCR amplified product of 618 bp for 16S rRNA gene of *Pseudomonas spp.* isolates, MWM - molecular weight marker (100 bp plus DNA ladder), + control (Positive, Negative) + different strains of *Pseudomonas* spp.

Conclusion

This study has shown that the rate of antibiotic resistance 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 patterns 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 therapeutics strategies.

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