

Detection of Multi drug resistance *Pseudomonas* in meat and milk samples from retail outlets of Chennai region

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Abstract

Pseudomonas, an opportunistic pathogen for humans is ubiquitous in nature and also a serious food spoilage organism important in food safety. The presence of this bacterium indicates external contamination which shows improper sanitation in the food chain. Recent findings substantiate that *pseudomonas* besides causing food spoilage, also helps in spread of some of the zoonotic microaerophilic organisms to flourish in foods of animal origin. In this study, 20 samples of each normal milk, refrigerated milk, chilled meat and 15 mastitis milk samples were examined for presence of *pseudomonas*. Prevalence of 25% and 35% was reported in refrigerated milk and meat, which indicates post processing contaminant as well as a spoilage organism. *Pseudomonas* plays a pathogenic role in 15 % of mastitis cases studied. Isolated strains were further studied for their sensitivity to antibiotics which again highlighted problem of resistance. This study revealed the presence of *pseudomonas* in foods of animal origin with multiple drug resistance which needs immediate attention of food industry.

Keywords: *Pseudomonas*, food safety, prevalence, antibiotic resistance.

Introduction

Pseudomonas, an aerobic gram negative, psychrophilic rod has a multi faced potential of causing harm to public. Naturally it is a ubiquitous organism causing opportunistic infection in humans and food spoilage in refrigerated foods. Psychrotrophic bacteria have become an escalating problem for the food industry after introduction of refrigerated storage of raw and processed foods. The main psychrotrophic microflora present in raw milk are Gram negative rods *Pseudomonas* spp. comprising at least 50% of the total bacteria in refrigerated foods (Uraz

and Citak, 1998). Most predominant organism that are often associated with meat spoilage is *Pseudomonas*. Besides being a serious food spoilage organism, *Pseudomonas* is a leading cause of nosocomial infections, ranking second among the gram negative pathogens reported to the National Nosocomial Infection Surveillance System. *Pseudomonas aeruginosa* is notorious for its resistance to antibiotics therefore; it is particularly dangerous and dreaded pathogen (Kenneth et al., 2011). Hence this study was planned to screen the refrigerated food for the presence of *pseudomonas* and know about their susceptibility to antibiotics.

Materials and Methods

Samples included in this study were raw milk, refrigerated milk and mastitis milk and meat samples. A total of 75 samples, 20 of each in normal, refrigerated milk, meat samples and 15 mastitis milk were analyzed. Raw milk and mastitis milk were collected from MVC teaching hospital. Refrigerated milk and meat samples were bought from retail market shops in and around Vepery region of Chennai. Each milk sample was mixed well and 0.1ml of milk samples (normal, refrigerated & mastitis) were inoculated in the 10ml sterile McConkey broth. Meat samples were cut into pieces and mixed with 225 ml of McConkey broth in stomacher bag. Inoculated broth was incubated at 37°C. The grown colonies were streaked in McConkey agar plates and incubated. Non sorbitol fermenting colonies (White colonies) with fruity smell were sub cultured on readymade *pseudomonas* agar plates (Himedia, Mumbai) and its duplicate was made in *pseudomonas* F agar plates and incubated. *Pseudomonas* isolates were confirmed by oxidase, citrate test. Antibiotic sensitivity of the isolates was studied by Kirby Bauer disc diffusion method. 500µl of the

broth was transferred in to the Mueller Hinton agar and the culture was distributed equally over the entire surface. Antibiotics discs used are tetracycline (10 µg), Enrofloxacin (10 µg), Gentamicin (10 µg), Amoxicillin sulbactam (10 µg +10 µg) and Amikacin(10µg).

Results and Discussion

On analyzing all the 75 samples, 14 samples were positive by culture, biochemical tests.

Table: 1 Isolates obtained from the samples

| Samples | No | Isolates | Prevalence | ABST |
|-------------------|----|----------|------------|------|
| Normal milk | 20 | 0 | - | - |
| Refrigerated milk | 20 | 5 | 25% | MDR |
| Mastitis milk | 15 | 2 | 13% | MDR |
| Chilled meat | 20 | 7 | 35% | MDR |

MDR- Multiple Drug Resistant- Isolates which have resistance against more than one drug.

| Samples | Isolates | Tetracyclin | Amoxicillin sulbactam | Gentamicin | Enrofloxacin | Amikacin |
|-------------------|----------|-------------|-----------------------|------------|--------------|----------|
| Refrigerated milk | RM1 | R | R | S | S | S |
| | RM2 | R | R | R | R | IS |
| | RM3 | IS | R | IS | S | S |
| | RM4 | R | R | S | S | S |
| | RM5 | R | R | S | S | IS |
| Mastitis milk | MM1 | R | R | R | IS | S |
| | MM2 | R | R | R | R | S |
| Chilled meat | CM1 | R | R | IS | S | S |
| | CM2 | R | R | S | R | IS |
| | CM3 | IS | R | IS | R | IS |
| | CM4 | R | R | S | S | S |
| | CM5 | R | R | R | S | S |
| | CM6 | IS | R | S | R | S |
| | CM7 | IS | R | S | S | S |

S - Susceptible

- >3mm zone of inhibition

IS - Intermediately Susceptible

- 1-3mm zone of inhibition

R - Resistant

- no zone of inhibition

Almost all isolates were showing 100% resistant to Tetracycline and Amoxicillin sulbactam. Isolates were resistant to Enrofloxacin and Gentamicin were 35.71% and 28.57%. All the isolates were sensitive only to Amikacin. It is evident from the results that the prevalence of Pseudomonas was noticed in the samples which are stored for sometime i.e Post processing contamination. Predominance of Pseudomonas in refrigerated milk is in accordance with the findings of Benna et al.,(2011). Post processing contamination was already revealed by MacPhee & Griffith (2002). Presence of Pseudomonas in meat is well studied and universal fact. Friederike Hilbert et al., (2010) has proved experimentally that pseudomonas in meat create microaerophilic atmosphere

which would favour zoonotic organism such as campylobacter to survive and affect humans. Problem highlighted by this study is multiple drug resistance. Beena et al.(2011) revealed Multiple drug resistant strains which were sensitive to Enrofloxacin. But in this study even the isolates are resistant to Enrofloxacin. This kind of resistance emergence is very common in pseudomonas due to its habit of forming biofilms in nature. Therefore detection and enumeration of pseudomonas is useful to track down the contamination sources and hygienic status of the stored foods. By controlling the presence and activity of pseudomonas, it is possible to extend the shelf life of foods and also in preventing biofilm formation which is the

main place of emergence and spread of resistance antimicrobial genes.

Summary

The presence of pseudomonas in stored foods and mastitis milk was analysed, and the antibiogram of the isolates were studied. The study highlighted two problems, one is 25 – 35% prevalence in refrigerated foods and 15% in mastitis, another is that currently evolving isolates have 100% resistance to tetracycline and Amoxycillin Sulbactam. 35.71% and 28.57%. and resistance to Enrofloxacin and Gentamicin and all the isolates were susceptible to Amikacin. So, immediate attention should be taken against the contamination of pseudomonas in cold chain maintenance as multidrug resistance to current use antibiotics has been evinced in this study and Pseudomonas being an ubiquitous organism can transfer the drug resistance genes to other pathogens also.

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