ANTIMICROBIAL RESISTANCE AN INTERFACE BETWEEN ANIMAL AND HUMAN DISEASES

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ABSTRACT

In recent years, concerns about the use of antimicrobial products in food-producing animals have focused on human food safety because foods of animal origin are sometimes identified as the vehicles of food borne disease in humans and, therefore, also vehicles of resistant food borne pathogens and resistant genetic material. The major zoonotic pathogens of concern for the development of antimicrobial resistance are Salmonella spp. and Campylobacter jejuni.. A broad spectrum of antimicrobials are in use in animals One such antimicrobial is Silver, used to treat a various infections . The current widespread and uncontrolled use of silver may result in more bacteria developing resistance, analogous to the emergence of antibiotic and biocide resistant bacteria. This could be very detrimental to many industrial and medicinal properties that depend on the microbial properties of silver. In the present study, in order to gain an insight into bacterial resistance to silver the clinical isolates of Salmonella typhimurium were plasmid cured, the antibiotic sensitivity was tested in order to find out whether the strains were drug resistant; the Plasmid cured bacterial strains were then grown in nutrient broth containing silver nitrate, in order to determine silver resistance. To identify the protein encoded by this silver resistance determinant, a whole cell lysate was made and an SDS-PAGE was carried out. Plasmid was cured .The results of the study showed that silver resistance is plasmid encoded. A low molecular weight protein found in wild strains was found missing in the plasmid cured strains and possibly could play role in silver resistance.

Key words: Zoonotic transmission, Salmonella typhimurium, Silver resistance

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INTRODUCTION

There is no field of human endeavor, whether it be in industry or agriculture or in the preparation of food or in connection with problems of shelter or clothing or in the conservation of human or animal health and combating of disease, where the microbe does not play an important and often dominant role (Pelzar, Micro organisms and their activities are increasingly central to many of the concerns of the society. They are closely associated with the health and welfare of human beings. Bacterial resistance to toxic agents is often found under conditions where toxicity might select for resistance.

Organisms of the genus *Salmonella* cause three distinct clinical syndrome (1) Gastroenteritis (2) Enteric fever (3) Septicemia. Salmonellosis is a major health problem throughout the world.The most common manifestation of *Salmonella* infection is enteritis and dissemination of bacteria outside of gastro intestinal tract is unusual in the healthy host.Multi drug resistance is a serious problem in the treatment of bacterial infections.Silver resistance is important to monitor because modern technology has developed a wide range of products that depend on silver as a key microbial component(Amita gupta et al, 1998).

The veterinary profession shares the concerns of the public, governmental agencies, and public health community regarding the broad issue of antimicrobial resistance and specifically the potential risk of resistance developing in animals with subsequent transfer to humans. Judicious use of therapeutic antimicrobials is an integral part of good veterinary practice. It is an approach to maximize therapeutic efficacy and minimize selection of resistant microorganisms (Merck vet manual.com, cdc.gov.com)

MATERIALS AND METHODS

Bacterial Culture

The ***Salmonella typhimurium*** strains used in the study are clinical isolates provided by Christian medical college ,Vellore.The strains were routinely sub cultured on nutrient agar at 37ºC.liquid cultures were grown in nutrient broth at 37ºC for 18 hours.for storage ,the strains were grown overnight in nutrient broth and stored at -20ºC after adding glycerol.

Plasmid Curing

Plasmid was cured by the method of Mark.S.Guyer and Alwin.J.Clark. [(4)].

Curing was done using acridine orange.5 ml of LB broth containing 5 µg of acridine orange / ml was inoculated with colonies less than 1000 cfu / ml.the broth was incubated for 96 hours. After the period of incubation, the bacterial colonies were streaked on nutrient agar plates so as to obtain individual colonies.The individual colonies were then picked using tooth pick and pricked into 2 sets of agar plates, one containing LB medium alone and the other containing LB media and suitable concentration of ampicillin.the plates were then incubated for 18 hours at 37ºC .The cured bacterial colony can then be selected.

Antibiotic Sensivity Test

The bacterial colonies were suspended in sterilized saline and a uniform

suspension was made. The bacterial suspension was then swabbed on nutrient agar plates. Antibiotic discs were then placed using sterilized forceps. The plates were incubated for 18 hours. The sensitivity of the bacteria towards the antibiotics were seen as zones around the discs.

Whole Cell Lysate

Bacterial colonies were scrapped and suspended in 10% hot SDS. The solution was vortexed and about 30 µl of lysozyme (1mg/ml) was then added. The solution was then centrifuged at 10,000 rpm for 15 minutes.The supernatant was taken for further analysis.To 30 µl of the supernatant equal amounts of sample solublising buffer was added and subjected to SDS-PAGE.

Separation of Outer Membrane Protein (OMP)

OMP was isolated by the method of Carolene et al.,(1986).One colony of bacteria was subcultured on Petri plate and incubated for 18 hours at 37ºC .The cells were scraped from agar and suspended in 0.01 M HEPES buffer.The cells were disrupted by sonication employing four 1 min pulses on ice with an interval of 1 min between the pulses. The lysed cells were then centrifuged at 16,000 rpm for 5 mins to pellet the cell debris. The supernatant was suspended in 1 % sodium lauryl sarcosinate using magnetic stirrer for 1 hour at room temperature. The sarcosyl insoluble membrane fraction was then pelleted by centrifugation at 20,000 rpm for 30 mins and washed in minimal volume of HEPES buffer. Stored in microfuge tubes at 4 ºC.The protein content of OMP preparation was determined by lowyy's method. Equal amounts (50 µg) of OMP from wild and plasmid cured strain was loaded on SDS-PAGE.

RESULTS AND DISCUSSION

Antibiotic Sensivity Test

The two clinical isolates of ***Salmonella typhimurium*** were tested for their antibiotic sensitivity. Isolate I (wild strain) was found to be resistant to chloramphenicaol, tetracycline, ampicillin, sulphamethizole, nalidixic acid (Table 1) (Fig. 1)

After being plasmid cured .isolate I was sensitized to all of the mentioned antibiotics . Similarly isolate II (wild strain) was found to be resistant to chloramphenicaol, ampicillin, sulphamethizole and nalidixic acid (Table 1).

After being plasmid cured isolate II was sensitized to all of the mentioned antibiotics (Fig. 2)

Table 1.
Antibiotic sensitivity test for *Salmonella typhimurium* Isolate I and II.

S.No	Antibiotics	Isolate I response	Isolate II response
1.	Ampicillin	Resistant	Resistant
2.	Amoxycillin	Resistant	Resistant
3.	Chloramphenicol	Resistant	Resistant
4.	Ceftazidime	Resistant	Resistant
5.	Cifroflaxin	Sensitive	Sensitive
6	Co-Trimoxazole	Sensitive	Sensitive
7	Nalidixic acid	Resistant	Resistant
8	Polymyxin	Sensitive	Sensitive
9	Streptomycin	Resistant	Sensitive
10	Sulphamethizole	Resistant	Resistant
11	Tetracycline	Resistant	Resistant

Isolates I and II were found to multi drug resistant. This antibiotic resistance poses a genuine problem worldwide since drugs like chloramphenicaol and ampicillin had been first line drugs for the treatment of invasive ***Salmonella*** infections. Transmissible multi drug resistance were transferred from donor to recipient strains and thus almost all bacteria become multi drug resistant (Pruitt et al., 1998)

Susceptibility of Salmonella To Silver nitrate

The two different clinical isolates of ***Salmonella typhimurium*** were plasmid cured .The wild and plasmid cured strains were then grown in silver nitrate containing medium. Wild strains showed more growth irrespective of the silver concentration, while plasmid cured strain showed a very less growth in silver containing medium (Table 2)

Table 2: Susceptibility of Salmonella to Silver nitrate

Organism	O.D at 600 nm.		Number of bacterial cells	
	0.5 mM Silver nitrate	**0.1 mM Silver nitrate**	**0.5 mM Silver nitrate**	**0.1 mM Silver nitrate**
***S.typhimurium* Isolate I (wild)**	0.352	0.494	$9.39x10^7$	$13.17x10^7$
***S.typhimurium* Isolate I (Plasmid cured)**	0.146	0.169	$3.89x10^7$	$4.57x10^7$
***S.typhimurium* Isolate I (wild)**	0.707	0.767	$28.28x10^7$	$30.68x10^7$
***S.typhimurium* Isolate I (Plasmid cured)**	0.554	0.630	$24.16x10^7$	$25.20x10^7$

The results suggest that silver resistance in ***S. typhimurium*** is plasmid encoded. Isolate I showed a significant decrease of 50 -60 % in silver nitrate containing medium after being plasmid cured while Isolate II showed a 30 % decrease.This shows that isolate I is significantly silver resistant while isolate II not much silver resistant (Table 3).

Table: 3 : Percentage decrease in the number of bacterial cells in plasmid cured strain when compared with wild strain

Organism	0.5 mM Silver nitrate	0.1 mM Silver nitrate
***S.typhimurium* Isolate I**	58.57%	65.30%
***S.typhimurium* Isolate I**	14.56%	31.30%

Human encounters with silver containing products are surprisingly numerous world wide, primarily as a biocide. The medical uses of silver have been documented since 1000 B.C.Silver resistant bacteria have been reported (Pruitt et al, 1998) and there have been incidences associated with invasive burn wound infections (Mc hugh et al, 1975). Until recently the molecular basis for bacterial resistance to silver was not known and even the conditions that optimally differentiate between silver resistance and silver sensitive growth were not clear (Levy et al., 1998). Resistance to silver is co-transferable from one strain of bacterium to another, the donor strains containing the silver resistance determinant transfers it gene (transmissible plasmids) to the recipient strains by conjugation (Mc hugh et al, 1975).

Whole Cell Lysate Profile of Wild and Plasmid Cured Strains

An attempt was made to identify the protein that was encoded by the silver resistant determinant. For this purpose, a whole cell lysate was made for both wild and plasmid cured isolates. A 15% SDS-PAGE was carried out. The results showed that a low molecular weight protein (approximately 14 Kilo Dalton) found in wild strain missing in the plasmid cured strain (Fig. 3).

Earlier studies showed that a protein of 14 Kilo Dalton is responsible for silver resistance in other pathogenic bacteria suggesting a homology among different pathogens for silver resistance.

Omp Profile of Wild and Plasmid Cured Strains

With a presumption that the protein encodede by the silver resistance determinant might be the outer membrane protein, the OMPs from wild and cured strains were isolated and a 10 % SDS-PAGE was carried out.

There were no significant changes in the OMP profile of the wild and cured strains. This suggest that the silver that the silver resistance determinant might code for some other protein and not necessarily the outer membrane protein.The silver resistance protein may be periplasmic as in other ***Salmonella*** species.

CONCLUSION

Salmonella bacteria are the most frequently reported cause of food borne illness.Animals are the ultimate source for all *Salmonella* infections.Emergence of Antimicrobial resistant bacterium poses serious threat to the society.Silver resistance is one among these.Current wide spread and uncontrolled use of silver may result in more bacteria developing resistance analogous to the emergence of antibiotic resistance in animals.Through Zoonotic transmission silver resistant bacterium finds way in to humans**.**

In this study we have identified that ***S.typhimurium*** strains got from Christian Medical College ,Vellore were found to be multi drug resistant .The drug resistance was found to be plasmid encoded.The clinical isolates were also found to have plasmid encoded silver reistance.The whole cell lysate and the OMP profile suggested that silver resistance protein might be periplasmic.

Fig. 1: Isolate I wild and Plasmid Cured strains

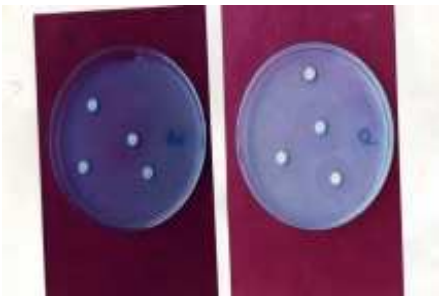


Fig. 2: Isolate II wild and plasmid cured strains

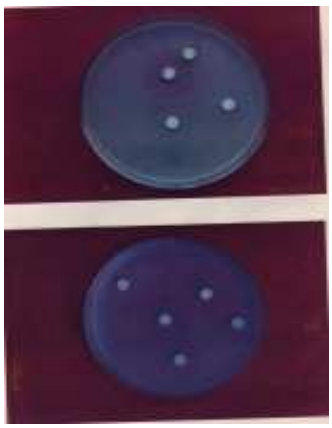
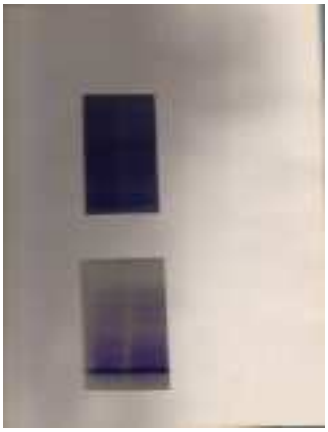


Fig. 3: Whole cell lysate Profile of wild and plamid cured strains



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