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ISOLATION AND CHARACTERISATION OF MULTI DRUG RESISTANCE STRAINS FROM SKIN OF COWRANCH WORKERS

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ABSTRACT

When drug resistance occurs, the drug or combination of drugs loses its ability to block the germ from reproducing. Over time, the treatment can stop working completely. This means the drugs become less effective. Various microorganisms have survived for thousands of years by adapting to drugs. For the isolation of potential MDR strains associated with both animals and humans, 36 different samples were collected from the Volunteers working in cowranch of nearby areas. From which 9 isolates were selected as MDR strains. These 9 isolates were used for further characterization on the basis of microscopic and biochemical tests. Isolates were assigned tentatively as genera as *Micrococcus*, *Streptococcus*, *Bacillus*, *Staphylococcus*.

Keywords: Antibacterial agents, Multiple drug resistance, Antibiotics, Isolates, Cowranch.

INTRODUCTION

Multi-drug-resistant (MDR) bacteria represent a major threat to human and animal health [1]. It is now almost commonplace to hear about methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), multi-drug resistance in *Mycobacterium tuberculosis* (MDRTB) strains and multi-drug-resistant (MDR) Gram-negative bacteria. So-called new and emerging pathogens add to the gravity of the situation [2]. Drug-resistant strains initially appeared in hospitals, where utilization of antibiotics was greatest. Multidrug-resistant (MDR) bacteria were detected in the late 1950s and early 1960s. Enteric gram-negative bacilli—*Escherichia coli*, shigella, and salmonella—were the first MDR bacteria identified [3]. MDR is not necessarily the result only of the treatment of the cells by a drug. Often it is connected with the type of cell differentiation or the localization of cells in an organism [4]. There are a number of mechanisms whereby bacteria can develop antibiotic resistance including horizontal transfer of resistance genes drug specific selection of naturally occurring resistant variants within a population, and increased mutagenesis in hypermutator strains [5,6]. Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs [7].

MATERIAL AND METHODS

For the isolation of potential MDR strains associated with both animals and humans, 36 different samples were collected from the skin of the Volunteers working in cowranch of nearby areas.

Isolation of microorganisms

For the isolation of microorganisms, 36 samples were collected from nearby areas like cow-yards and animal houses. Samples were collected in sterile swab tubes and brought to laboratory. Isolation of bacteria was preceded on nutrient agar plates by directly streaking the samples. Inoculated plates were incubated at 37^0C for 24 hours in incubator followed by purification. Purification was done by picking the colonies from petriplates and re-streaking on the fresh nutrient agar petriplates and again

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incubated under similar set of conditions. This procedure was repeated till pure culture was obtained. After that pure colonies were picked and inoculated into the sterile broth test tubes. Test tubes were incubated at 37^0C for 24 hours. Pure cultures were then preserved at 37^0C for antimicrobial sensitivity test and further experiments.

To check the antimicrobial activity of isolates against drugs i.e. Susceptibility test procedure

Mueller-Hinton agar plates were prepared. The medium in the plates was sterilized. The entire agar surface of plates was spread with culture. Now antibiotic discs were placed aseptically. After 15 minutes plates were inverted and placed in the incubator at 37^0C. Examined each plate after 20-24 hours of incubation. If plates were satisfactorily spread the resulting zone of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Accuracy of test depends upon the disc potency, proper inoculums, functional pretested medium plates (nature of medium and its depth), inoculation technique, incubation temperature and time, etc. To maintain the potency of discs, the stock containers of discs were stored in the freezer at -20^0C. Removed the antimicrobial discs from refrigerator to room temperature 1-2 hours before use to avoid moisture condensation. The zone diameter of zone was measured to the nearest whole millimeter at the point at which there is prominent reduction in growth.

Morphological characterization

Morphological characterization shows the appearance of microorganisms under the microscope on a slide and culture characteristics on agar plate and in broth medium

Microscopic Characteristics

Microscopic characteristics tell about the shape and staining characteristics of the strain. These observations noticed with the help of microscope.

(a) Shape:-Spheres (cocci), rods, filaments, commas, spirals.

(b) Gram stain: - Positive (+ve), negative (-ve).

Biochemical characterization

Biochemical characterization is done by proceeding some tests, as indole production test, MRVP test, citrate utilization test, starch hydrolysis test, catalase test, hydrogen sulphide production test, gelatin hydrolysis, casein hydrolysis, urease test, fermentation of carbohydrates and nitrate reduction tests [8].

RESULTS AND DISCUSSION

Testing of isolates against different antibiotics discs-Antimicrobial agents susceptibility test

Antibiotics used for susceptibility tests were Amoxicillin (AMX), Bacitracin (B), Ceftazidime (CA), Cefepime (CPM), Imipenem (I), Levofloxacin (LE), Piperacillin/Tazobactam (PTZ), Methicillin (MET), Oxacillin (OX), Kanamycin (K), Cefotaxime (CE) and Ciprofloxacin (CF).

While using discs the results were interpreted as-

Sensitive: If the zone was more than the given standard

Resistance: If the zone was less than the given standard.

Intermediate: If the zone was fallen between the above limits given by CLSI standard

Zone scale measurement of MDR isolates against different antibiotic discs

In the present study MDR status was assigned to those isolates where zone of inhibition was less than or equal to the standard zone of inhibition recommended by CLSI.2009 on the basis of zone scale measurement (Table 1).

Morphological characterization

Selected isolates were subjected to Gram's staining to confirm their inherited morphological characters and metabolic machinery. Strains MS-2(rods), MS-11(Coccus), MS-12(Coccus), MS-14(Coccus), MS-15(Coccus), MS-19(Rods), MS-21(Rods), MS-22(Coccus), MS-25 (Coccus) were Gram positive under compound microscope.

Table 1. Zone scale measurement (mm) of MDR isolates against drugs

Sr. No.	Sample No.	Drugs											
		CA .03 mg	PT .1/.01 mg	B .01 mg	CF .03 mg	CE .01 mg	I .01 mg	MET .005 mg	LE .005 mg	AMX .03 mg	OX .001 mg	K .03 mg	CPM .03 mg
1	MS2	6	15	6	30	7	13	6	37	23	9	16	6
2	MS11	6	17	6	18	14	22	7	20	19	7	23	14
3	MS12	6	18	6	28	12	22	7	19	19	6	8	11
4	MS14	6	18	6	26	13	13	6	27	12	6	13	12
5	MS15	6	16	6	24	12	23	6	23	11	7	16	8
6	MS19	6	18	6	27	13	13	6	18	16	6	21	6
7	MS21	6	17	6	31	9	19	6	14	19	6	18	9
8	MS22	6	21	6	28	8	20	6	13	13	6	21	11
9	MS25	6	16	6	29	12	17	6	12	12	6	19	30

Table 2. Biochemical characterization of different MDR isolates

Sr . No.	Test Name	MDR Isolates								
		MS 2	MS 11	MS 12	MS 14	MS 15	MS 19	MS 21	MS 22	MS25
1	Staining	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
2	Starch Hydrolysis	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
3	Urease test	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
4	Indole test	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve
5	Methyl Red test	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve
6	VP test	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
7	Casein test	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
8	Catalase test	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
9	Gelatin liquefication test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
10	Hydrogen sulphide test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
11	Nitrate test	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
12	Citrate test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
13	Carbohydrate Fermentation test:									
(a)	Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
(b)	Lactose	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
(c)	Sucrose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Biochemical Characterization

Selected MDR strains were characterized in terms of their biochemical potential for different substrates and these results were tabulated in Table 2.

These 9 isolates were tentatively assigned genera as MS-2 was *Bacillus* belongs to *Bacillaceae*, family, MS-11 was *Streptococcus* belongs to family *Streptococcaceae*, MS-12 and MS-14 were *Staphylococcus* belongs to family *Staphylococcaceae*, MS-15 was *Micrococcus* belongs to *Micrococcaceae*, MS-19 and MS-21 were *Bacillus* belongs to family Bacillaceae, MS-22 and MS-25 were *Staphylococcus* belongs to family *Staphylococcaceae*.

CONCLUSION

In this study isolation and characterization of MDR strains were done by collecting the 36 samples from cow ranch of nearby areas. Antimicrobial susceptibility test was performed on MHA plates. Out of 36 samples, 9 isolates were identified as MDR. Percentage resistance of microorganisms against different antibiotics is 34% for first generation, 30% for second, 34% for third and 19% for fourth generation antibiotics. On the basis of morphological and biochemical tests, isolate MS-15 was identified as *Micrococcus.* Isolates MS-11 was identified as Streptococcus. Isolates MS-2, MS-19 and MS-21 were identified as Bacillus. Isolates MS-12, MS-14, MS-22 and MS-25 were identified as *Staphylococcus.*

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