
RESISTOGRAM AND ANTIBIOGRAM TYPING OF *CLOSTRIDIUM CHAUVOEI*

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Many chemical compounds exert toxicity with in species of micro organisms. This character can be used to define a profile of a strain based on its resistance to selected compound and such a profile of the strain is referred to its “resistogram”. Resistotyping and antibiogram are simple, quick, reproducible methods and can be incorporated easily into daily bench routine. Black quarter is one of the most important economically devastating bacterial diseases encountered in cattle. Pathogenic *Clostridium chauvoei* has been incriminated as the main causative agent of black quarter in cattle. This paper describes the usefulness of resistotyping and antibiogram method for the differentiation of *Clostridium chauvoei* and its potential value in epidemiological studies.

In this study, four isolates of *Clostridium chauvoei* were isolated from cases of black quarter in cattle from different places of Tamilnadu.

Resistogram

The isolates were subjected to resistogram and antibiogram typing as described by Elek *et al* (1973)

Stock solutions of dyes and chemicals were used at the concentrations shown in table 1 and were designated by a letter.

Clostridial selective agar plates were prepared. One control and one each with respective compounds were added. Three ml of each chemical in two different concentrations added to 27ml of agar was found to be more effective in discriminating the strains and that ratio was followed for all the compounds. The strains were grown overnight at 37°C in anaerobic gaspak system in 1 ml Clostridial selective broth in 10ml screw capped bottles and before use diluted to 1 in 10 by the addition of 9ml of sterile distilled water. The diluted overnight culture was inoculated by means of Takatsy microtitre loop. This provided the requisite inoculum size of about 1,000 organisms which in the control plate should result in confluent growth. Before inoculation the plates were dried for 20 minutes at 37°C. Each of the 10 plates was then inoculated and a reference point was marked for subsequent reading. The spots of inocula were allowed to disappear and the plates were kept at 37°C

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incubation for 18 hours. The plates were incubated anaerobically in anaerobic gaspak system as described by Awaad *et al*(1980).

Antibiotic Sensitivity Testing

Antibiotic Sensitivity test was carried out as per the method of Bauer *et al* (1966) with slight modifications. The top surface of 3 to 5 colonies of the test organism were used as inoculum in 5 ml of brain heart infusion broth and incubated at 37 °C degree celsius overnight anaerobically. The growth was diluted with normal saline to a density visually equal to McFarland 0.1. Brain heart infusion agar plates were dried in the incubator at 37°C for 30 minutes before inoculation. The diluted bacterial growth was streaked in 3 different plates on brain heart infusion agar using a sterile swab. The plates were dried at room temperature for 5 minutes followed by placing discs of antibiotic(Himedia) by means of a sterile forceps. Seven discs were placed equidistant hexagonally in each petridish of 15 cm diameter and such plates were placed used for each test organism. After overnight incubation 37°C for 24 hrs, the diameter of each zone was measured and interpreted according to the zone size as per the interpretation charts.

Resistotyping

The resistogram study revealed that 100% resistance was observed with minimal concentration

(0.5w/v) of coppersulphate, acriflavine, boric acid and phenylmercuric nitrate for all the four isolates where as only 25% resistance was observed for brilliant green.(Table1) The result of this study is found to be in accordance with. Elek *et al* (1973)

Antibiogram

The antibiogram patterns of the four isolates of *clostridium chauvoei* of black quarter origin were determined. The multiple antibiotic resistance exhibited by four isolates of *Clostridium chauvoei* to 7 antibiotics are presented in Table2. All the four isolates showed 75% resistance to norfloxacin, 50% of isolates exhibited resistance to penicillin and doxycycline. The resistance to these antibiotics might be due to indiscriminate usage in day to day practice as reported by Kulshreshtha *et.al*(1974). All the four isolates were 100% sensitive to the antibiotics ciprofloxacin and enrofloxacin in correlation with the findings of Verma(1998). Antimicrobial susceptibility was used as an epidemiological marker to study dissemination of multi resistant *Clostridium chauvoei*.

The results suggest that the drug of choice for black quarter due to *Clostridium chauvoei* is ciprofloxacin and enrofloxacin followed by oxytetracycline, gentamycin, penicillin and doxycycline in the order of sensitivity.

Table 1

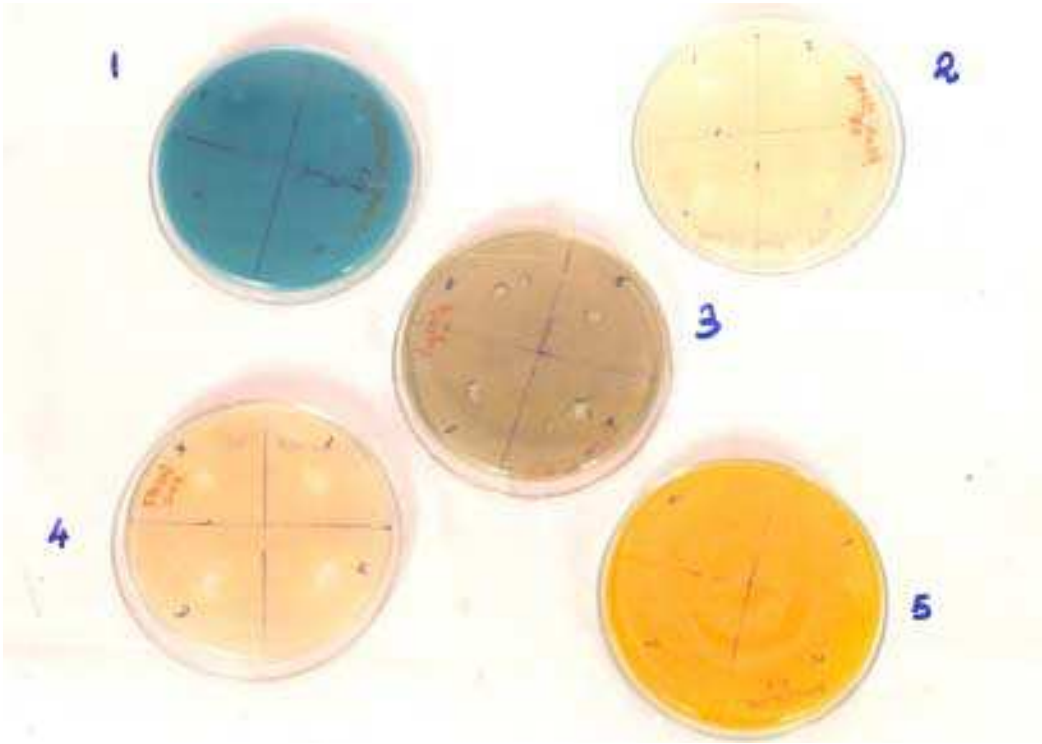
Percentage resistance of four *Clostridium chauvoei* isolates of Black quarter origin to dyes and chemicals

Reference Letter	Antibacterial dyes and chemicals.	Concentration (% w/v)	Number of isolates Resistant	Percentage of Resistant
A	Brilliant Green	0.5	1	25%
		1.5	0	0%
B	Copper Sulphate	1.5	4	100%
		2.0	0	0%
C	Acriflavine	0.2	4	100%
		0.3	2	50%
		6.0	4	100%
D	Boric acid	7.0	2	50%
		0.01	4	100%
E	Phenylmercuric nitrate	0.05	2	50%

Table 2

Antibiogram of *Clostridium chauvoei*

A
C
E
I
Ge
Ox
I



Resistotyping

1. Brilliant Green
2. Boric acid
3. Copper Sulphate
4. Phenyl mercuric Nitrate
5. Acriflavine