The Isolation and Bio-chemical Identification of Clostridium tetani

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Abstract: The Clostridium tetani is causative agent of tetanus, spastic paralysis, a vaccine preventable disease, caused by the second most poisonous substance known, the tetanus toxin (TetX). Tetanus is more remarkable and globally prevalent disease of human and vertebrate animals. The estimated worldwide deaths from tetanus were 213,000 in 2002 including 198,000 in children under 5 years of age including neonatal tetanus This study aimed for isolation of causative agent of tetanus, C.tetani its animal and biochemical testing along with the antimicrobial susceptilbility. The achievements of present study was isolation of tetanus causative agent by clinical identified tetanus patients and from deep puncture wound.

Keywords: C.tetani, Cephalosporins , Haemolysis , NIH, Obligate anaerobe, PIMS, Tetanus,.

Introduction:

Clostridium tetani is an obligate anaerobe, cosmopolitan, readily endospore, and Gram positive bacillus (Izurieta *et al*., 1997; Farrar *et al*., 2000; Kenneth, 2004). Mostly present in habitat like soil, dust, and intestinal tracts of various animals, horses, chicken and humans (Holger *et al*., 2002; Allen *et al*., 2002; Schwartz *et al*., 1990). *C. tetani* spores are ubiquitous, and able to withstand many environmental factors, and comparatively resistant to disinfectants and desiccation (Wilkins *et al*., 1988). Usually spores are introduced into body by wounds, cuts, and burns, and once become carried into wounds it may not germinate instantly, and may become active after the wound has healed (; Bleck *et al*., 1997). This organism is the causative agent of tetanus, spastic paralysis, a vaccine preventable disease (Udwadia *et al*., 1994; World Health Organization; www.who.int_vaccines diseases_diseases_ Neonatal Tetanus. shtml) caused by the second most poisonous substance known, the tetanus toxin (TetX), with a human lethal dose of 1ng/kg (Hara *et al*., 1977; Holger *et al*., 2002). The WHO definition of adult tetanus requires in any case one of the subsequent signs: trismus (inability to open the mouth) or risus sardonicus (sustained spasm of the facial muscles); or painful muscular contractions. Even if this definition requires a history of injury or wound, tetanus may also occur in patients who are incapable to remember a specific wound or injury (Who Technical Note, 2010). With reference to cure, Penicillin was the standard therapy for tetanus in most parts of the world (Sanford *et al.,* 1995; Johnson *et al.,* 1945; Ahmadsyah *et al*., 1985; Yen *et al*., 1997). Metronidazole is now considered the first line of therapy and is a safe alternative to penicillin (WHO Technical Note, 2010; Sanford, 1995; Ahmadsyah *et al.,* 1985). Tetracyclines, macrolides, clindamycin, cephalosporins and chloramphenicol are also effective (WHO Technical Note., 2010; Abrutyn, 1995).

Methodology:

Study design: The study was performed in National Institute of Health (NIH), Islamabad 100 samples were taken from wound swabs of patients and blood from diagnosed tetanus patients over a 6-month period, from Pakistan Institute of Medical Sciences (PIMS) and most wounds were generally deep puncture tissue injuries.

Microbiologic culture and characterization: Blood agar plates were used to analyze the morphology of ***C.tetani***. Cultural blood agar plates were made by homogenization of culture with agar at the time of pouring of media in plates.

Biochemical tests: H_2S, DNAse, gelatin liquifies, nitrate reduction, starch hydrolysis, lipase and lecithinase activity tests were done. All biochemical tests was performed on fresh isolates only, to avoid any discrepancies that may occur as a result of long term storage.

Antimicrobial Susceptibility: Disc sensitivities were performed using penicillin (10units), metronidazole (5µg), chloramphenicol (30µg), erythromycin (15µg), sulfamethoxazole (25µg), Ciprofloxacin (75µg) and ofloxacin (5µg) mostly prescribed antibiotics were placed on the solidified agar plates.

Results:

Morphology:

The bacterial strain *Clostridium tetani* was confirmed through different morphological and biochemical tests. As about 100samples were collected, only five samples (three from tetanus diagnosed patient and two from wound samples) were positive to *C. tetani*. It produced a thin transparent film of swarming growth on the blood agar surface. Blood in blood agar plates showed haemolysis. In Gram stained smear of culture, showed some vegetative cells stained as gram-positive rods but sporing rods

showed the typical round, terminal, distending spores (i.e. **'drumstick'spores**) stained as gram-negative rods.

Biochemical Tests:

Biochemically the strain was identified as H_2S and DNAse producing species, was also able to liquefies gelatin, but showed negative reactions for nitrate reduction, starch hydrolysis, lipase and lecithinase activity.

Antimicrobial Susceptibility:

Table 01 (Figure 1) shows the results of different antibiotics applied on *C. tetani* from different samples of patients. An antibiotic E showed a clear zone of about 01cm in A,02, and H samples of *C. tetani,* while ≤0.1cm zone showed in **C.tetani** Aii and 19 samples. Mz and SXT antibiotics showed a clear zone of 01cm in *C. tetani* A,AII and 19 samples, while ≤0.1cm zone were cleared by samples of *C. tetani* 02 and **C.tetani** H samples illustrated the 01cm zone with Mz antibiotic, while 0.7cm zone with SXT antibiotic. 0.9 cm clear zone were shown by *C. tetani* A against T antibiotic, ≤0.1 cm zone was shown by **C.tetani** Aii and 19, while *C. tetani* 02 showed 0.1 cm and **C.tetani** H 0.2 cm zone against antibiotic T. Against samples *C. tetani* Aii and 19, detected zone was ≤0.1cm zone with antibiotic P, while 0.7cm zone, against *C. tetani* 02 and ***C.tetani*** H showed 0.5cm zone with antibiotic P, while sample *C. tetani* A showed 0.1cm cleared zone, with mentioned antibiotic P, Detected zone against OFX of samples *C. tetani* A, Aii, 02, H, were 01 cm. While ≤0.1cm zone was detected by **C.tetani** 19. 0.7 cm zone was shown by samples **C.tetani** A and Aii and detected zone by **C.tetani** 19 was ≤0.1, while 01cm by sample **C.tetani** 02, and 0.5cm by **C.tetani** H sample, against antibiotic C.

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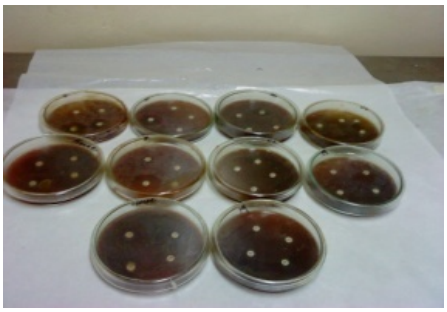

(Figure 1)

Samples	Antibiotics						
	E (cm)	MZ (cm)	CFP(cm)	T (cm)	P (cm)	OFX (cm)	C (cm)
C.tetani A	01	≤0.1	≤0.1	0.9	0.1	01	0.7
C.tetani Aii	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	01	0.7
C.tetani 19	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1
C.tetani 02	01	01	01	01	0.7	01	01
C.tetani H	01	01	0.7	0.2	0.5	01	0.5

Table 01