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Prevalence of Antibiotic Resistant Enterobacteriaceae in Medical Gauze as Unseen Environmental Pollutant

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Abstract: The present study was undertaken to screen the bacterial isolates causing hemolysis present in medical gauze, used to wrap wounds. The medical gauze was obtained from medical store Anand, Gujarat, India. Isolation was done according to Clinical Laboratory Standard Institute (CLSI) guidelines along with 16S rDNA characterization and further PCR was performed to check the presence of hemolysin gene. In all, 10 different bacterial colonies were isolated out of which seven were found to be resistant against one or the other antibiotic. Molecular characterization of colony number 7 confirmed the presence of hemolysin gene and was found to be resistant to large number of antibiotics, identified as *Enterobacter hormaechei*.

Enterobacter hormaechei is lesser known strain as not much has been reported in India. But this is thought to be one of the major bacteria responsible for nosocomial infection by means of various transmissions. Medical gauzes are widely used in surgeries and covering wounds. The possible presence of antibiotic resistant bacteria like this can give rise to devastating effect leading to various nosocomial outbreaks.

Key words: Nosocomial, medical gauze, *Enterobacter hormaechei*.

Introduction

Medical gauzes are white bleached cloth which are mainly made up of cotton and widely used for the wound care dressing. According to United States Pharmacopeia (USP), absorbent gauze is either cotton or mixture of cotton but not more than 53% by weight of rayon. Also it must meet the standards of purity, thread count, manufacturing and sterility according to USP (USP, 29). Medical gauzes are routinely used in all the hospitals and primary health centres all over the world to cover the wounds and protect them from the environment (Morgan, 2015). Therefore the level of purity, sterility and packaging of the medical gauze must be done very carefully (Rolstad et al., 2012). Any error in sterility or packaging may lead to contamination of the gauze which ultimately leads to the chances of secondary infections in the wound area which might be difficult to treat if the infection is caused by multidrug resistant (MDR) species (Hess, 2013).

Development of antibiotic resistance in bacteria is rigorously growing phenomenon nowadays. After the discovery of penicillin, antibiotics had saved millions of lives and had transformed human health. However, their excessive usage has made them ineffective. This is mainly because of the development of various resistance patterns in the bacteria for the antibiotics (Stuart and Bonnie, 2004). Incidences of nosocomial infections or hospital acquired infection caused by bacteria have also increased since 1980s which mostly includes the species of *Staphylococcus*, *Enterococci*, *Pseudomonas* and *Mycobacteria* (Banerjee et al.,

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1991). *Enterobacteriaceae* is considered as a major causative agent for nosocomial infections normally present in clinical and non-clinical samples as widely spread contaminant. Excessive usage of third and fourth generation cephalosporins had made certain *Enterobacteriaceae* species to produce ESBL and AmpC making them more vulnerable to treat (Paterson, 2006). Evidences are still meagre for an alternative treatment for the following, as everyday large numbers of antibiotic resistant bacteria are emerging; henceforth researches and investigations are on processes; if ignored, may lead to a scarcity of antibiotics in coming times.

The objective of this study was to isolate pathogenic bacteria from medical gauze resistant to wide array of antibiotics.

Materials and Methods

Sample Collection

Medical gauze was collected from medical store near Vallabh Vidya Nagar, Anand, Gujarat, India. The gauze used in present study was of absorbent type, 2′ × 2′ and average mesh size of 1.5 mm. The samples were collected in a sterile, zip locked polythene bag and carried to the laboratory for further processing.

Isolation of Bacterial Flora

Medical gauze was cut into pieces, placed in 5 ml nutrient broth and incubated for 24-48 hours at 37°C. Thereafter, the broth cultures were plated on selective and/or differential media for the characterization of bacterial isolates. The isolated colonies obtained on nutrient agar medium were then characterized on the basis of colony morphology, cellular morphology, staining and other biochemical parameters using standard microbiological techniques (Godkar, 2007). Futher the zone of clearance on blood agar plate was studied to confer the hemolytic activity of the each bacterial isolate.

Antibiotic Sensitivity Test

All the isolates were tested against seven different antibiotics namely Streptomycin, Vancomycin, Cefoxitin, Ampicillin, Tetracycline, Penicillin and Nitrofurantoin for antibiotic susceptibility testing (CLSI, 2011).

Amplification of α-Hemolysin (*Hly-α*) Gene

The DNA was isolated by bacterial DNA isolation kit (Xcelgen). PCR was carried out in a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube in Eppendorf Thermal Cycler. The PCR protocol designed for 30 cycles for the primers used; is given in Table 1 (Wang et al., 2011).

Table 1: Steps and conditions of thermal cycling for PCR (hlyα-gene)

Steps	*Temperature*	*Time*	*Cycles*
Initial denaturation	95°C	3 min	1
Final denaturation	94°C	60 sec	
Annealing	60°C	45 sec	30
Extension	72°C	60 sec	
Final extension	72°C	10 min	1

Primer Detail

HLYA-F 5’ GAAGTCTGGTGAAAACCCTGA 3’
HLYA-R 3’ TGAATCCTGTCGCTAATGCC 5’

Bacterial Identification Using 16S rDNA Amplification

The colony was subjected to automated DNA sequencing on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). Sequencing was carried out using Big Dye®Terminator v3.1 Cycle sequencing kit following manufacturer’s instructions. The 16S rDNA gene sequence was compared with the nrdatabase of NCBI genbank database using BLAST. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4.

Results

In all 10 different bacterial isolates were obtained from the medical gauze. Initially, the colony characteristics of all the 10 bacteria were studied, as shown in Table 2. All the isolates were further subjected for various biochemical tests (Table 3). Pathogenic characteristics of the isolates were studied by observing zone of clearance on blood agar plates. Colony no C1, C3 and C9 were showing beta hemolysin production which was confirmed by observing clear zone around the colonies, while colony C2, C5, C6, C7, C8, C9 and C10 showed alpha hemolysin production which was confirmed by observing the greenish zone of RBC lysis around the colonies. The antibiogram result has been shown in Table 4, which explains that the strain was resistant to variety of antibiotics like Cefoxitin, Penicillin-G, Vancomycin and Streptomycin. Here colony 7 was found to be resistant to all the classes of antibiotics used in this study. As colony 7 showed good hemolytic activity as well as resistance against

Table 2: Colony characterization of isolated strains

Characterization	*C1*	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
Size	Small	Medium	Small	Large	Medium	Medium	Medium	Medium	Small	Large
Shape	Round	Rod	Elliptical	Round	Round	Rod	Rod	Rod	Irregular	Round
Margin	Entire	Undulate	Undulate	Entire	Entire	Entire	Entire	Entire	Entire	Articulate
Texture	Smooth	Smooth	Rough	Rough	Rough	Smooth	Smooth	Rough	Rough	Rough
Pigmentation	Pale greeen	No	Pale yellow	Pale yellow	Pale yellow	No	No	Pale yellow	No	No

Table 3: Result of biochemical test

Tests	*C1*	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
Coagulase test	-	-	-	-	-	-	+	-	-	-
Citrate utilization test	+	+	-	+	-	+	+	-	-	-
Catalase test	-	-	-	-	-	-	-	-	-	-
H_2S production	-	+	-	-	-	+	+	+	+	+
Phenylalanine test	-	-	-	-	-	-	-	-	-	-
Indole test	+	+	-	-	+	+	-	-	-	+
Methyl red test	-	+	+	-	+	+	+	-	-	-

+ Positive; - Negative

four classes of antibiotics, it was selected for further characterization. Figure 1 explains the growth of colony 7 on various solid media. Colony 7 showed positive citrate utilization test and methyl red test which confirmed the isolate belonging to *Enterobacteriaceae* family. PCR amplification of the **α–Hemolysin gene** revealed the presence of 700 bp **α–Hemolysin** gene to be present on the chromosomal DNA of the bacterial isolate. 16S rDNA sequencing showed 1369 bp DNA amplification. Comparison of the consensus sequence of C7 with nrdatabase of NCBI genbank revealed the isolate as *Enterobacter* species specifically *Enterobacter hormaechei*. In each case the maximum identity was found to be 100% (Figures 2, 3 and 4).

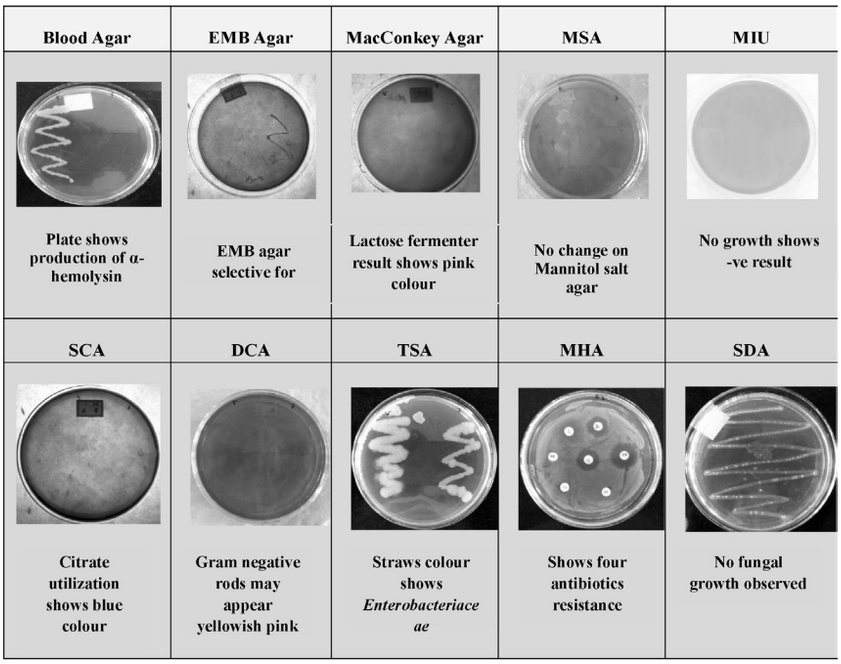

Figure 1: Colony 7 on solid media.

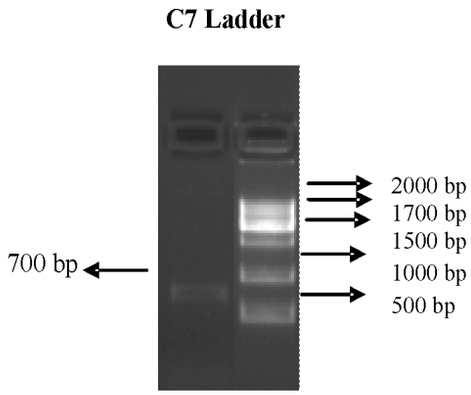

Figure 2: PCR amplification of *hly*-α gene.

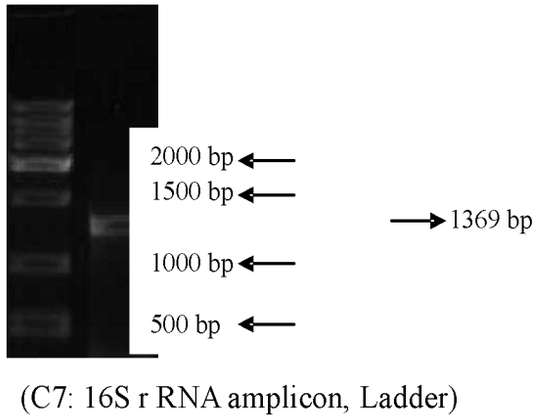

Figure 3: Gel image of 16S rDNA amplicon.

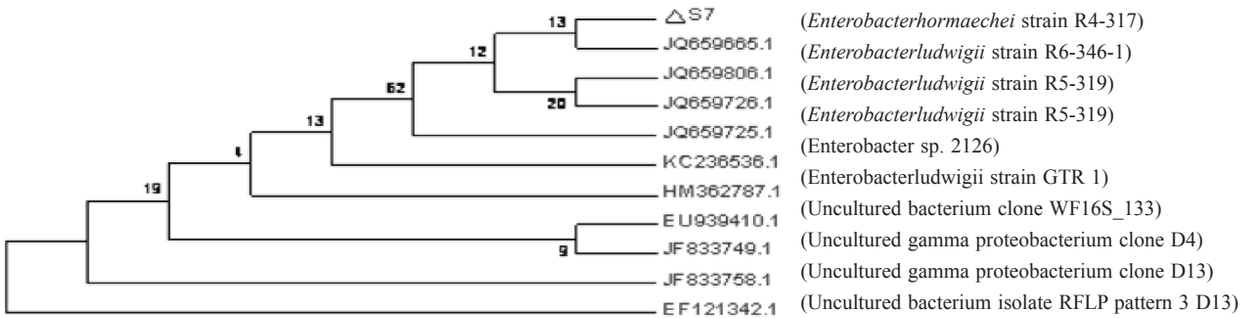

Figure 4: Phylogenetic tree of the isolate.

Table 4: Antibiogram of all the ten isolates

Tests	*C1*	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
Ampicillin	S	S	S	S	S	S	S	S	S	R
Tetracycline	S	S	S	S	R	S	S	S	S	R
Cefoxitin	S	S	S	S	S	S	R	S	S	S
Penicillin-G	S	S	S	S	R	S	R	S	S	R
Vancomycin	S	S	S	S	R	S	R	S	S	S
Streptomycin	S	S	S	S	R	S	R	R	S	R
Nitrofurantoin	S	S	S	S	S	S	S	R	S	S

S – Sensitive; R – Resistance

Discussion

The isolated culture labelled as C7 was found to be *Enterobacter hormaechei* strain-R4-317 (GenBank Acc. No: JQ659665.1). The entire nucleotide homology and phylogenetic analysis was done on the basis of results obtained from different parameters, tests, staining properties and 16S rDNA sequencing.

Enterobacter hormaechei is found to be a new species belonging to enteric group which consists of 23 strains, 22 of which were isolated from humans (Hoffman and Stindl, 2005). *E. hormaechei* is a nosocomial pathogen that can infect immune-compromised patients in the hospitals that can be transmitted from patient to patient when infection control techniques are insufficient (O'Hara et al., 1989). It was first identified as a unique

species in 1989. Between Nov 1992 and Mar 1993, an outbreak of *E. hormaechei* occurred among premature infants in the intensive care nursery (ICN) at the hospital of the University of Pennsylvania (Garner and Simmons, 1983). *Enterobacter hormaechei* is also associated with extended spectrum beta-lactamase production, which further complicates the treatment by limiting therapeutic agents (Hurrel et al., 2008).

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