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EMERGENCE OF VRE IN A CVTS ICU: AN ACTIVE SURVEILLANCE AND MOLECULAR ANALYSIS

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ABSTRACT

Introduction- We report an epidemiological survey of Vancomycin Resistant Enterococci (VRE) in Cardiovascular Thoracic Surgery (CVTS) Unit of tertiary care hospital. The survey was conducted after we isolated VRE, (the first case of VRE from our institute) , from blood culture of a patient of a 50 year-old man who presented with signs and symptoms of sepsis on 4th post-operative day of mitral valve replacement.

Methodology- A set of Bactec blood culture from a patient grew *Enterococcus faecium* and *Candida tropicalis*. The *Enterococcus faecium* turned out to be Vancomycin resistant on vancomycin Bile aesculin agar (6ug/ml vancomycin) which was confirmed by doing MICs on (PHOENIX B.D) automated identification system.(MIC > 16 µg/ml) and Vancomycin E strip and Broth Microdilution method (MIC > 256 µg/ml). The rectal swab and antecubital swabs for VRE colonization were collected from this patient and 15 patients admitted during this period in the CVTS ICU and CVTS ward along with clinical details. The health care workers were also screened by collecting hand swabs. DNA-DNA hybridization was done to know similarity of index case isolate to that of VRE isolated from gut and skin of other patients.

Results- Out of 9 ICU patients, 4 were colonized with VRE and 5 with Vancomycin Sensitive Enterococci (VSE) in gut. Out of 6 patients in pre-operative ward all were colonized with VRE. DNA hybridization result showed high percentage similarity with index strain in 5 isolates from colonized patients. Prolonged perioperative stay > 2weeks and use of broad spectrum antibiotics like cephalosporins were found to be significant risk factors for VRE Colonization. HCWs were negative for VRE colonization. The outbreak was contained by thorough cleaning and fumigation of ICU, preoperative, post operative wards and common utility area like bathroom. All VRE colonized patients were discharged and prescribed probiotics for 3weeks for gut deconatamination.

Conclusion- Vancomycin resistant enterococci are emerging in hospital environment and are responsible for colonization and outbreaks of serious hospital acquired infections.

KEYWORDS : VRE, CVTS unit, colonisation

Introduction-

Vancomycin Resistant Enterococcus (VRE) is becoming an important cause of nosocomial infections. Plasmid mediated resistance to glycopeptides like vancomycin and teicoplanin was found in enterococci in 1988.[1]Since then VRE have caused cluster of nosocomial infections [2].Intensive Care units have been known to be foci of emergence and spread of VRE as these patients have been exposed to antibiotic therapy, hospital environment for a long time [3]. To prevent and control outbreaks due to VRE, active surveillance and strengthening of infection control practices are highly recommended strategies. [4]

In the present study we report a case of post-operative VRE in our institute from a patient in cardiovascular-Thoracic unit of our hospital. It presented as post mitral valve replacement bacteraemia. After this index case an intensive epidemiological surveillance was undertaken to know the colonization among other patients and also in ICU environment. Following the first case of VRE infection, and the subsequent increase in cases of colonisation, an active surveillance programme was implemented.

Methodology-

This was an active prospective survey conducted in department of Microbiology of a tertiary care teaching hospital. The CVTS ICU of our hospital is a 12 bedded ICU. VRE was isolated from blood culture of a 50 year male operated for mitral valve replacement . The patient presented with signs and symptoms of sepsis on 5th postoperative day. This being the first case of VRE from our institute an active surveillance was conducted to assess the role of hospital environment and colonisation in patients and Health Care Workers (HCW).

The skin swabs and rectal swabs were collected from index case and other patients admitted in CVTS unit (9 patients in ICU and 6 patients in CVTS pre-operative ward).Our hospital is a tertiary care hospital catering to urban as well as rural population from interiors. The patients admitted in pre-operative wards were from remote parts of the state. So they were kept for pre-operative fitness and dental antibiotic prophylaxis in the pre-operative wards only. The samples collected from patients comprised of rectal swabs and ante cubital swabs to know the gut and skin colonization by VRE.Demographic and clinical data was collected from these patients to find out correlation between VRE colonization and risk factors like age, sex, preoperative stay, immunocompromised status, intake of any preoperative antibiotics. Swabs were also collected from hands of health care workers. All these samples were inoculated on Bile aesculin agar containing 6ug/ml of vancomycin. VRE which were isolated on this agar were confirmed by conventional methods [5] and BD-Phoenix automated system. The MIC of all these isolates was done by using Vancomycin E strips (HIMEDIA Labs) and later confirmed by broth microdilution method using vancomycin powder (HIMEDIA). The surveillance samples were also collected from different areas of CVTS OT which included air settle plates, swabs from hand wash solutions, wash basins, Cidex solution, syringe pumps, suction machine.

All VRE isolates were stored and sent it to Agarkar Research Institute

Pune for molecular analysis. The isolates from the index case and other patients were tested by DNA-DNA hybridization method to know the similarities between DNAs.

Procedure [5]- The DNA from the isolates were extracted by in house Silica gel method. DNA concentration was determined initially (before shearing) by measuring the absorbance at 260 nm,and the DNA was sonicated in 2_ SSC buffer using a Branson sonifier 450 (Danbury, Connecticut, USA) for 10–14 pulses at the lowest setting (1–2), at an output of 50%.DNA incompletely sheared using the sonifier was further fragmented using the sonifier or an ultrasonic bath to get more uniformly small-sized segments, optimally about 400– 1500 bp. Experiments were done with sheared DNA of approximately 0.1 mg/mL, resulting in a final DNA concentration of 1 mg/well. Sheared DNA samples in 2_ SSC buffer were added in 10 mL aliquots (single) or two 5 mL aliquots for DNA pairs to a 384-well PCR plate (Applied Biosystems). Ten microlitres of SYBR Green I nucleic acid stain (Invitrogen, Carlsbad,

California, USA) (SG) diluted 1 : 5 000 or 1 : 10 000 in 2_ SSC was added to each well for a total final volume of 20 mL and the contents were pipetted up and down once to ensure mixing. The preparation of the SG solution and addition to the plate was done under low light conditions and the plate was vortexed (10 s) and centrifuged briefly prior to melting and reassociation of the DNA in the 7900HT Fast Real-time PCR System. After the initial start at 25. 8C (5 s) and an increase to 99. 8C (100% ramp), the temperature was maintained at 99. 8C 10 min .After DNA

melting, the temperature was decreased quickly to 55, 60, or 70. 8C and maintained at that temperature for 40 min for reassociation to take place. After that, the temperature was again reduced (100% ramp) to 25. 8C and held there for 30 s. Fluorescence readings were taken every 7–10 s throughout the protocol.Using the Applied Biosystems software (SDS 2.3) associated with the 7900HT, the fluorescence readings were exported as text files, and those measurements recorded during the renaturation step were plotted against time in minutes,and linear regression trend lines were fitted. The slopes of the linear regression lines, representing the renaturation rates expressed as the increase in fluorescence per minute, *v*', were used to determine the degree of binding (%). The isolates showing high percent similarity with index isolate were considered related to each other i.e. they could be from a common source

Results-

VRE was isolated from blood culture of a patient who was a case of post mitral valve replacement bacteraemia. This isolate was resistant to vancomycin with MIC > 256 mcg/ml and was sensitive to linezolid. The swab collected from antecubital fossa and rectal area of this index patient also grew VRE with same susceptibility pattern. This indicates that the patient was colonized with VRE. Based on sensitivity report, patient was started on I.V.linezolid and Voriconazole. Out of 9 patients admitted in ICU during same period, we could grew VRE from 4 patients while 5 grew Vancomycin Sensitive Enterococcus (VSE). Out of 6 patients in pre-operative ward all grew VRE. The hand swabs collected from health care workers (including doctors (n= 5), nurses (n=13) and wardboys n=4) did not grew VRE. Also surveillance samples collected from CVTS OT and ward were negative for VRE.

Table 1- Results of DNA- DNA hybridization (n=11 =10 colonosiers +one index strain)

No	Isolate	Vancomycin susceptibility	% hybrid
1	*E.faecium*	Resistant	Index strain
2	*E.faecium*	Resistant	**102**
3	*E.faecium*	Resistant	**88.9**
4	*E.faecium*	Resistant	**101.9**
5	*E.faecium*	Resistant	41.5
6	*E.faecium*	Resistant	**123.5**
7	*E.faecium*	Resistant	**83.07**
8	*E.faecium*	Resistant	**206.0**
9	*E.faecium*	Resistant	63.59
10	*E.faecium*	Resistant	**162.2**
11	*E.faecium*	Resistant	77.0

DNA- DNA hybridization result (table 1) showed that, 6 strains from colonized patients (2 from ICU and 4 from pre-operative ward) showed high percentage hybridization with index strain.

Out of 15 patients 14 were in the age group of 30-60years and Male: Female ratio was 2.7:1. All 15 patients had preoperative stay > 2weeks and were on amikacin and cephalosporin as preoperative antibiotic prophylaxis. The preoperative patients with gut colonization were prescribed probiotics (yoghurt) for 3 weeks and discharged. They were asked to come back again after 3 weeks after gut decontamination.

Discussion-

In the present study VRE was isolated from blood culture of a patient with nosocomial bacteremia. This was the first case of VRE reported from our institute. Lucas et al also reported VRE bacteremia from 93 out of 210 patients in their study [6] while Peta et al had reported VRE from urethral discharge of a patient in ICU [3]. Boyce et al isolated VRE from 37 patients in their study.[7] Silverman et al has reported 0% vancomycin resistance amongst community acquired enterococci emphasizing its importance as hospital acquired bug [8]

Following the index case of VRE, vigorous epidemiological survey was conducted to look for VRE colonizers in ICU. In the present study VRE 66% (10 out of 15) were VRE colonizers. Peta et al has reported 75% VRE colonizers in ICU patients. [3] Gombarotto et al reported in 26.7% faecal VRE colonizers in hematology unit.[9]. A very low percentage of VRE colonization has been reported in other European studies from Netherland 2% [10], France 4.9% [11] and Belgium 3.5% [12]

All the VRE isolates showed very high MICs to vancomycin (MIC>256 mcg/ml) and were sensitive to linezolid.In the study by Peta et all the isolates were sensitive to Linezolid and Quinpristin-dalfopristin.[3]

The risk factors for VRE colonization in our study were prolonged pre-operative stay (> 2 weeks) and exposure to broad spectrum antibiotics like 3rd generation cephalosporins. Lucas et al reported prolonged hospitalization and metronidazole exposure as risk factor for VRE colonization.[6]

DNA hybridization study showed high percentage similarity between index isolate and 6 isolates from colonized patients (2 from ICU and 4 from pre-op ward. This indicates horizontal transmission of VRE across the patients.

The source for this outbreak could be common utility area between pre-operative and post-operative patients like bathroom. So thorough cleaning and fumigation of bathroom, ICU, preoperative and post operative wards were done to contain the outbreak. All preoperative patients were discharged and prescribed yogurt for 3weeks for gut decontamination.

Conclusion: Prolonged perioperative stay and use of broad spectrum antibiotics like cephalosporins may be responsible for VRE colonization. Hence achievable preventive measures should be taken to prevent such outbreaks from occurring in future.

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