Front. Biol. 2015, 10(6): 520–527
DOI 10.1007/s11515-015-1380-0

RESEARCH ARTICLE

Community and hospital acquired methicillin resistant *Staphylococcus aureus* efficiently retain the *Van A* determinant

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Abstract **Dissemination of vancomycin resistance from hospital to community strains is a serious threat to public health. Our study aimed to provide evidence for transmission of *Van A* type resistance from the hospital to the community. Wild-type community and hospital associated methicillin resistant *Staphylococcus aureus* strains were studied *in vitro* and in a model that mimicked a natural environment to ascertain their ability to acquire and maintain the vancomycin resistance determinant (*Van A* gene) from vancomycin resistant *Enterococcus faecalis*. Fitness was assessed and the cost of Van A acquisition and retention was estimated. *In vitro* mating experiments were carried out using a filter mating technique and a model of a natural water body environment. Transfer of resistance was carried out in two different conditions: restricted and favorable. Transconjugants were confirmed by E test and PCR using specific primer sets. Growth kinetics and fitness measurements were done by spectrometric analysis. Using the *in vitro* filter mating technique, high transfer frequencies that ranged from 0.7×10^{-3}(0.0006) to 3.1×10^{-4}(0.00011) were recorded, with the highest transfer frequencies for CA MRSA (thermosensitively homogenous) (0.7×10^{-3}), and 1.2×10^{-4} to 2.4×10^{-6} in the model. HA MRSA (homogenous) showed a greater capacity (3.6×10^{-4}) to receive the *Van A* gene, while CA MRSA showed a reduced ability to maintain the gene after serial subcultures. CA and HA thermosensitively heterogeneous MRSA transconjugants exhibited higher growth rates. The present study provides evidence for the enhanced ability of CA and HA MRSA clones to acquire and maintain *Van A* type resistance.**

Keywords *Van A* resistance, MRSA, fitness, growth kinetics, resistance transfer

Introduction

Infections with methicillin resistant *Staphylococcus aureus* (MRSA) and its rapid dissemination into the community have posed a serious threat to public health infrastructure globally. Hospital associated (HA) and community associated (CA) MRSA are differentiated on the basis of their SCC*mec* type. To date, 11 SCC*mec* types have been reported (International working group). An understanding of the rapid dissemination of antibiotic resistance is further complicated in HA and CA MRSA by the frequent interactions between community and tertiary healthcare centers. This problem has increased undesirable antibiotic pressure in hospitals. Hence, it is of immense value to understand the flow of drug resistance from hospitals to the community, particularly in those strains that were not previously drug resistant. Vancomycin is used as alternative treatment for nosocomial infections caused by MRSA. However, the increase in vancomycin use has resulted in the emergence of two major types of glycopeptide-resistant *S. aureus*: vancomycin intermediate-resistant *S. aureus* (VISA) which is characterized by a thickened and poorly cross-linked cell wall and leads to the accumulation of acyl-D-alanyl-D-alanine (X-D-Ala-D-Ala), and vancomycin-resistant *S. aureus* (VRSA), which has acquired the *Van A* operon from *Enterococcus* spp. carried by transposon Tn*1546* and causes high-level resistance (Arthur et al., 1993; Arthur et al., 1996; Cui et al., 2003). The first report of high-level resistance to glycopeptides appeared in the late 1980s in *Enterococcus* spp. (Leclercq et al., 1988; Uttley et al.,1988; Hiramatsuet al., 1997). The rapid dissemination of this resistance to other bacteria, including MRSA clones, is a matter of utmost concern (Howden et al., 2010). Seven types of vancomycin resistance determinants have been reported, namely *Van A*, *B*, *C*, *D*, *E*, *G*, and *L*, corresponding to their specific operons in the genome, plasmid, or transposons. The

Received October 3, 2015; accepted December 2, 2015
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most common and high-level glycopeptide resistance governing the *Van A* operon is inducible and transferred by a transposon carrying the resistance determinant designated Tn*1546* (Arthur et al., 1993; Chang et al., 2003; Courvalin et al., 2006). Horizontal gene transfer by conjugation and plasmid transfer is considered to be the most significant means of drug resistance transfer in the environment (dela Cruz and Davies, 2000).

The worldwide emergence of VISA has been documented in previous studies (Courvalin, 2006). To highlight the ability to spread vancomycin resistance from hospitals to the natural environment, the present study focused on determining the strength of *Van A* type gene transfer, evaluating the stability of the gene transferred to a new host, and comparing the growth kinetics between the donor *E. faecalis* and recipient HA and CA MRSA clones under *in vitro* and natural conditions. Considering that the dissemination of vancomycin resistance to the environment can result into a major public health problem, we evaluated the fitness of transconjugants of both CA and HA MRSA clones. The phenotypic expression of methicillin resistance has been shown to be under the control of factors like temperature (Annear, 1968); hence, we investigated the effect of temperature on the phenotypic expression of the *Van A* determinant. Thus, the entire set of experiment was carried out at two temperatures, 30ºC and 37ºC.

Methods and materials

Characteristics of bacterial strains

Community acquired methicillin resistant *S. aureus* strains were from Dr. A. B. Bhatt's Laboratory (these strains had been isolated from healthy individuals from the community who had not been to the hospital for at least 2 months prior to the study, which was conducted in 2012 by Kainthola and Bhatt). Hospital acquired methicillin resistant *S. aureus* strains were also isolated from patients with confirmed HA MRSA infections at Veer Chandra Singh Garhwali Medical College and Hospital. All strains were subjected to biochemical characterization and SCC*mec* A gene typing by multiplex PCR to confirm the identity of the strains. Strains with SCC*mec* type IV a, were considered to be community acquired and types I, II, III were considered to be hospital acquired.

S. aureus strains obtained from community and hospital sources were subjected to methicillin resistance phenotype analysis under two temperatures, 30ºC and 37ºC, and were categorized in four major groups: a subpopulation harboring the staphylococcal chromosomal cassette *mec* (SCC*mec*) type IV gene and showing resistance to methicillin at 30ºC (thermosensitive heterogeneous CA MRSA, which was able to grow in the presence of methicillin at 30ºC) and at 37ºC (thermosensitive homogenous CA MRSA), and strains harboring the SCC*mec* type III gene and showing resistance to methicillin at 30ºC (thermosensitive heterogeneous HA MRSA) and at 37ºC (thermosensitive homogenous HA MRSA) (Hartman and Tomasz, 1986). In total, four strains were used for the study, with one strain representing each group.

The donor strain used to transfer the vancomycin resistance *Van A* gene was *E. faecalis* JH2-2, while the recipient strain used was wild-type CA MRSA with SCC*mec* type IVa that was sensitive to vancomycin.

Inoculum preparation

Active cultures of CA MRSA were grown overnight at 37°C in broth media supplemented with oxacillin at a concentration of 4 μg/mL. *S. aureus* ATCC 29213 was used as a control strain to determine the MIC. The donor *E. faecalis* JH2-2 strain was grown in the same manner, but with vancomycin at a concentration of 8 mg/L. Two colonies from these cultures was picked and grown in 25 mL of non-selective broth. The recipient heterogeneous CA MRSA strains were grown with oxacillin in tryptone broth at 30ºC and the non-thermosensitive CA MRSA strain was grown at 37ºC. After incubation overnight, a 100 μL aliquot from each of these was inoculated into fresh broth (without any antibiotics) and incubated for 4 h to reach the mid-exponential phase of growth. A cell suspension was recovered by centrifugation at 5000 *g* for 10 min at 4°C, washing and centrifugation in maximum recovery diluent (MRD) (CM733; Oxoid), and, finally, dilution in 3 mL MRD. One milliliter each of both the recipient and donor strains was used as the initial concentration in mating experiment experiments.

Resistance transfer

Bymating

A filter-mating procedure (Klare et al. 1992) was used for the vancomycin-resistance transfer experiment Transconjugants were selected on brain heart infusion (BHI) agar (Oxoid) plates containing 32 mg of vancomycin /mL (Eli Lilly).

Mating in a model mimicking the environment

Natural environmental conditions, with optimum substrates (favorable) and without defined substrates (restricted), were mimicked in a laboratory setup to estimate the strength of vancomycin resistance gene transfer in a natural environment. Two different polycarbonate-walled 15 cm × 30 cm × 15 cm batch reactors were prepared in the laboratory. The floor of the system was made of stainless steel with a 3 cm bed of soil. The system was filled with water from the Alaknanda river (a river near the hospital) and 5g/1000 mL of peptone. The system was attached to an automated pulsed waterbath heater, which maintained the temperature at 37ºC and 30ºC in the two systems. Continuous 5% CO_2 was supplied by an inlet

rubber pipe. Both batch reactors were then inoculated with 2 mL of donor and 2 mL of recipient cultures prepared as described earlier. For the control, the same apparatus and same ingredients were used, but without the donor culture. Finally, the reactors were incubated for 24 h and 100 μL of each dilution (up to 10^{-7}) was spread plated onto selective agar containing 16 μg/mL of vancomycin. Colonies that appeared after 48 h were enumerated and replica plated onto Baired Parker agar medium so that only CA MRSA transconjugants could develop and be recognized. The experiment was repeated five times in triplicate and measurements were taken every 12 h. Colonies grown on the plates were counted and the efficiency of gene transfers, estimated as the number of transconjugants per donor cell, was calculated.

Confirmation of transconjugants

Screened transconjugants obtained after mating were cultured for 12 h in broth with vancomycin, then subsequently were streaked onto nutrient agar plates containing vancomycin at different concentrations and incubated separately at 30ºC or 37ºC overnight. Transconjugants were subjected first to vancomycin free broth medium followed by nutrient agar plates with vancomycin. The test was applied to assess the stability of the antibiotic resistance marker. The test was repeated three times and MICs for the transconjugants and donor under vancomycin pressure were determined by an Etest strip (AB Biodisk, Solna, Sweden).

PCR detection of *Van A*

PCR conditions were as described elsewhere (Foucaultet al. 2009). The primers for amplification of *Van A* were: Forward TAATAGAACAAAAGTTGGC and Reverse TCACCCTT-TAACGCCGATA. The amplification conditions were an initial denaturation at 98ºC for 10 s, annealing at 50ºC for 1 min, polymerization at 72ºC for 1 min, and final extension at 72ºC for 5 min. The expected amplicon size was 1.2 kb with vancomycin resistant *E. faecium BM4147* as positive control strain.

Fitness measurements

Growth rates were determined by a spectrophotometer (Bio-Rad) reader (Labsystems). Donors, recipients, and selected transconjugants were grown overnight at 37°C or 30°C, with or without 8 μg/mL of vancomycin in broth medium. Hence, 6 cultures were subjected to fitness measurements. The cultures were diluted to reach the optical density of 0.15 OD into 20 mL of broth with or without 8 μg/mL of vancomycin and incubated at 37°C or 30°C in a shaker incubator. After 12 h of incubation, all of the cultures were diluted to 1:1000, inoculated into 200 μL of broth in a 96-well microplate, and then incubated overnight at 37°C and 30ºC with shaking. Absorbance was measured at 600 nm every 3 min. The relative growth rate for each strain was calculated as the ratio of the growth rate of the transconjugant or wild-type resistant strains vs. susceptible strains.

Analysis of vancomycin resistant subpopulations (population analysis)

The cell subpopulations resistant to vancomycin were analyzed as described elsewhere (Matsuo et al., 2011). Cultures were diluted and spread onto BHI agar plates containing vancomycin that ranged in concentration from 0 to 10 mg/L with a subsequent increase to 1 mg/L. After 48 h of incubation at 37ºC or 30ºC, the colonies were counted. Control strains used were VISA strain Mu50 and vancomycin susceptible *S. aureus* strain FDA209P.

Doubling time

The doubling time for each strain was determined as described elsewhere (Matsuo et al., 2011).

Results

Transfer frequency for horizontal gene transfer (HGT) in the filter mating technique

The filter mating technique was applied to determine the capacity for gene transfer between mating pairs *in vitro*. High transfer frequencies were recorded for all of the four mating pairs; however, the transfer frequency between CA MRSA (thermosensitively homogenous, *Van A*$^-$) and *E. faecalis* (*Van A*$^+$) was highest at 0.7×10^{-3}, followed by CA MRSA (thermosensitively heterogeneous, *Van A*$^-$) and *E. faecalis* (*Van A*$^+$). However, the HA MRSA strains lagged a little behind CA MRSA in acquiring the resistance gene *in vitro*. The average values of transfer frequencies for all mating pairs are shown in Table 1.

Table 1 Transfer frequency was calculated as the number of transconjugants obtained per donor cell

Characteristics of mating pair (donor + recipient)	Transfer frequency (Avg.)
CA MRSA (hom. *Van A*$^-$)+ *E.faecalis* (*Van A*$^+$)	0.7×10^{-3} (0.0006)
CA MRSA (hom. *Van A*$^-$)+ *E.faecalis* (*Van A*$^+$)	0.8×10^{-3} (0.0013)
CA MRSA (hom. *Van A*$^-$)+ *E.faecalis* (*Van A*$^+$)	3.1×10^{-4} (0.00011)
CA MRSA (hom. *Van A*$^-$)+ *E.faecalis* (*Van A*$^+$)	1.4×10^{-4} (0.00009)

Values are the averages of three independent replications, with the standard deviations stated in parentheses. Hom.; homogenous, het.; heterogenous, *Van A*; vancomycin A gene.

Transfer frequency for HGT in the environmental mimicking model

A model mimicking natural environmental conditions was prepared in the laboratory with different substrate conditions,

i.e., favorable and restricted. Substrate conditions are assumed to vary in different geographical locations. Mating pairs were then grown on the model to assess the resistance gene transfer frequencies. HA MRSA (thermosensitively homogenous, *Van A*$^-$) and *E. faecalis* (*Van A*$^+$) showed the highest rates of transfer when the substrate conditions were favorable and restricted (at 3.6×10^{-4} [0.00031] and 1.2×10^{-4} [0.00019]) followed by CA MRSA (thermosensitively heterogeneous, *Van A*$^-$) and *E. faecalis* (*Van A*$^+$) at 2.3×10^{-4} in favorable conditions. Transfer rates varied for all mating pairs greatly. Substrate conditions and transfer frequencies are shown in Table 2).

Further, once it was confirmed that the model explained gene transfer in natural environments or water bodies like river, ponds, and pools, all mating pairs were subjected to the environmental model, with transfer frequencies recorded at an interval of every 12 h (in favorable conditions), to assess the rates of gene transfer at various times. A gradual increase in gene transfer was observed in all the mating pairs. Table 3 depicts the increase in transfer rates over the time for all pairs, with the highest being CA MRSA (thermosensitively homogenous, *Van A*$^-$) and *E. faecalis* (*Van A*$^+$), ranging from 0.4×10^{-3} at 12 h of incubation to 1.4×10^{-2} at 36 h. The doubling time was estimated for control and donor strains and for transconjugants, showing that the doubling time for transconjugants increased markedly in comparison with their parental strains (Table 4). On comparing the doubling time for all strains, hospital associated transconjugant strains, in comparison with their community MRSA counterparts, showed a great increase in doubling time. The HA MRSA (homogenous) transconjugant took 44 min to double, while its parental HA MRSA (homogenous) strain took 29 min, showing a 15 min delay, followed by the HA MRSA (heterogeneous) transconjugant, which showed a 14 min delay in doubling time (Table 4).

Population analysis of the control and transconjugants

A population analysis was performed to determine the stability and strength of transferred genes under antibiotic pressure in a broth medium. Hospital associated MRSA transconjugants (both thermosensitively homogenous and heterogeneous) showed strong resistance against vancomycin, up to a concentration of 11 mg/L, whereas the community associated MRSA transconjugants, maintained resistance at higher vancomycin concentrations to almost constant CFU/mL (log10) (Fig. 1), even though they did show abrupt resistance at the vancomycin concentration of 1mg/L to 4 mg/L.

Growth kinetics after HGT

The initial inoculum was kept as low as 10^5 CFU to allow the detection of small differences in the lag phases of the different strains. It is evident from Fig. 2A and 2B that gene transfer interrupted the growth of transconjugants, signifying their compromised fitness. The thermosensitively heterogeneous transconjugants of both CA and HA MRSA had a little higher growth rate than the homogenous transconjugants.

Discussion

To understand the ability of enterococci to transfer vancomycin resistance and ability of CA and HA MRSA to receive, harbor, and maintain antimicrobial resistance determinants in a natural environment is of utmost importance to determining their contribution to the dissemination of resistance from hospitals to community settings or natural environments. Here, we report on the ability of the wild-type vancomycin resistance determinant *Van A* in *E. faecalis*, isolated from the gut of a patient suffering from colon ulcer, to be transferred to two clones of MRSA, i.e., community associated and hospital associated at 30ºC and 37ºC. The two temperatures were used to investigate the thermosensitivity of the resistance gene operon, as this has been described by various investigators in case of SCC*mec*. Previous studies have documented the transfer of the *Van A* gene cluster from *Enterococcus* species to *S. aureus in vitro* and in *in vivo* (Noble et al., 1992; Miller et al., 2002; Sievert et al., 2002; Kacicaet al., 2004; Weigel et al., 2007; Aligholi et al., 2008; Saha et al., 2008). All mating pairs showed the transferred resistance determinant in *in vitro* mating; however, using a conventional filter mating technique, it was observed that CA MRSA strains were received the transferred resistance determinants at higher frequencies than their HA counterparts (average transfer frequency of 0.8×10^{-3}). Temperature did not affect the transfer rate. CA MRSA clones are usually less resistant to antibiotics and, hence, are

Table 2 Average transfer frequency in mimicked model

Pairs (donor + recipient)	Conditions in mimicked system	Transfer frequency (average)
CA MRSA (hom. *Van A*$^-$) + *E. faecalis* (*Van A*$^+$)	River water + peptone + minerals + galactose + shaking incubation	2.4×10^{-6} (0.0000012)
	River water + shaking incubation	1.1×10^{-6} (0.0000002)
CA MRSA (het. *Van A*$^-$) + *E. faecalis* (*Van A*$^+$)	River water + peptone + minerals + galactose + shaking incubation	2.3×10^{-4} (0.00031)
	River water + shaking incubation	1.0×10^{-6} (0.0000013)
HA MRSA (hom. *Van A*$^-$) + *E. faecalis* (*Van A*$^+$)	River water + peptone + minerals + galactose + shaking incubation	3.6×10^{-4} (0.00031)
	River water + shaking incubation	1.2×10^{-4} (0.00019)
HA MRSA (het. *Van A*$^-$) + *E. faecalis* (*Van A*$^+$)	River water + peptone + minerals + galactose + shaking incubation	1.7×10^{-5} (0.000022)
	River water + shaking incubation	$2.9 \times \times 10^{-5}$ (0.000025)

Hom.; homogenous, het.; heterogenous, *Van A*; vancomycin A gene.

Table 3 Transfer frequencies on time scale from mimicked (at optimum condition) model

Mating pair	12 hours	24 hours	36 hours
1	0.4×10^{-3}	0.9×10^{-3}	1.4×10^{-2}
2	0.6×10^{-4}	0.5×10^{-4}	0.9×10^{-3}
3	1.2×10^{-5}	1.1×10^{-4}	2.4×10^{-4}
4	0.9×10^{-4}	0.5×10^{-4}	1.2×10^{-3}

1: CA MRSA (hom. *Van A*$^-$) + *E. faecalis* (*Van A*$^+$), 2: CA MRSA (het. *Van A*$^-$) + *E. faecalis* (*Van A*$^+$), 3: HA MRSA (hom. *Van A*$^-$) + *E. faecalis* (*Van A*$^+$), 4: HA MRSA (het. *Van A*$^-$) + *E. faecalis* (*Van A*$^+$).

Table 4 Effect of horizontal gene transfer (HGT; filter mating technique) on doubling time

Strains	Van MIC (mg/L)	Doubling time (min)
CA MRSA (hom.) transconjugant	8	39
CA MRSA (het.) transconjugant	8	37
E. faecalis (donor)	8	42
HA MRSA (hom.) transconjugant	8	44
HA MRSA (het.) transconjugant	8	46
Control strains	–	29
CA MRSA homogenous		28
CA MRSA heterogenous	–	31
HA MRSA homogenous	–	29
HA MRSA heterogenous	–	32

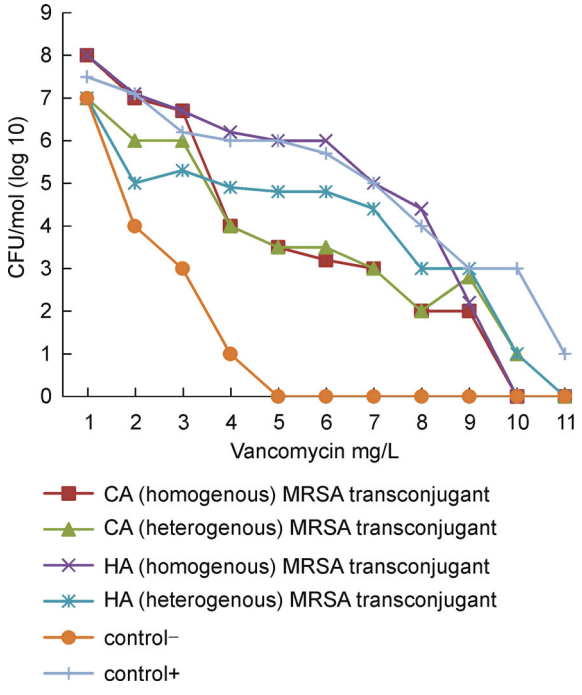

Figure 1 Population analysis of transconjugants and control strains.Control strains used:control + = VISA strain Mu50; control- = vancomycin-susceptible *S. aureus* strain FDA209P.

comparatively fitter to receive such determinants. However, maintenance of this resistance was compromised when the clones were sub cultured many times (Fig. 3).

The core concept of the study was to look for evidence that natural environments like water bodies are well suited for such gene transfers. Hence, we mimicked natural environments with favorable and restricted conditions and investigated the average transfer frequencies at various time. Earlier studies reported conjugal plasmid mediated transposon transfer of *Van A* type resistance to the *S. aureus* (Tenover et al., 2004; Zhu et al., 2008; Périchon and Courvalin, 2009); however, we have reported the difference in the abilities of two clones that differ genetically as well as phenotypically in MRSA resistance to acquire the gene from *E. faecalis* in environmental mimic model. Under favorable conditions (with added nutrients) HA MRSA (homogenous) showed greater capacity to receive the gene, followed by CA MRSA (heterogeneous) (at 3.6×10^{-4} and 2.3×10^{-4}, respectively). In the present study, the frequency of transconjugants obtained in the model ranged between 1.2×10^{-4} and 2.4×10^{-6}, which is quite low when compared to the *in vitro* filter mating technique, where the transfer rates ranged between 0.7×10^{-3} and 3.1×10^{-4}. Arguably, the model is a very different environment than *in vitro*, as the organic content, macro and micro nutrients, and other substances present in river water may hinder gene transfer, and mating *in vivo* involves many undefined biotic and abiotic factors that are not present in the conventional *in vitro* filter mating technique, where mating conditions are artificially optimized. Hence, it is not feasible to infer any direct relationship between these two systems in terms of gene transfer frequency. However, the difference in transfer rates was minimal, underscoring the potential problem of vancomycin resistance in real environment conditions. The transfer frequencies gradually increased in all of the mating pairs with time (Table 3). In the future, an investigation of how long this transfer can occur, as the gene was suppressed or deleted after subculturing many times, indicating that the sudden acceptance of a gene was followed by the rationale use of that gene by the cell, according to the environment in which it lives (Fig. 3).

Vancomycin resistance in enterococci is associated with the ability of bacteria to grow in the presence of vancomycin in the culture medium. Our findings are concurrent with this concept (Fig. 3). Enterococci strains with such peculiar properties have been isolated *in vitro*, in animal models, and from patients treated with vancomycin (Van Bambeke et al., 1999; Farrag et al. 1996; Kirkpatrick et al. 1999). Acquisition of *Van A* type vancomycin resistance by CA and HA MRSA thermosensitively heterogeneous transconjugants has been shown experimentally to result in better growth under vancomycin-added culture medium (Fig. 2A, 2B). Notably, thermosensitively homogenous CA and HA MRSA transconjugants lagged a little behind in terms of growth, showing that, at 30°C, the burden for expressing the SCCmec and *Van A* genes was mitigated by cell. Expression of co-resistance may be better understood *in vivo*; however, our puzzling findings suggest the expression of co-resistance by MRSA isolates when in the human body (at 37°C) and when

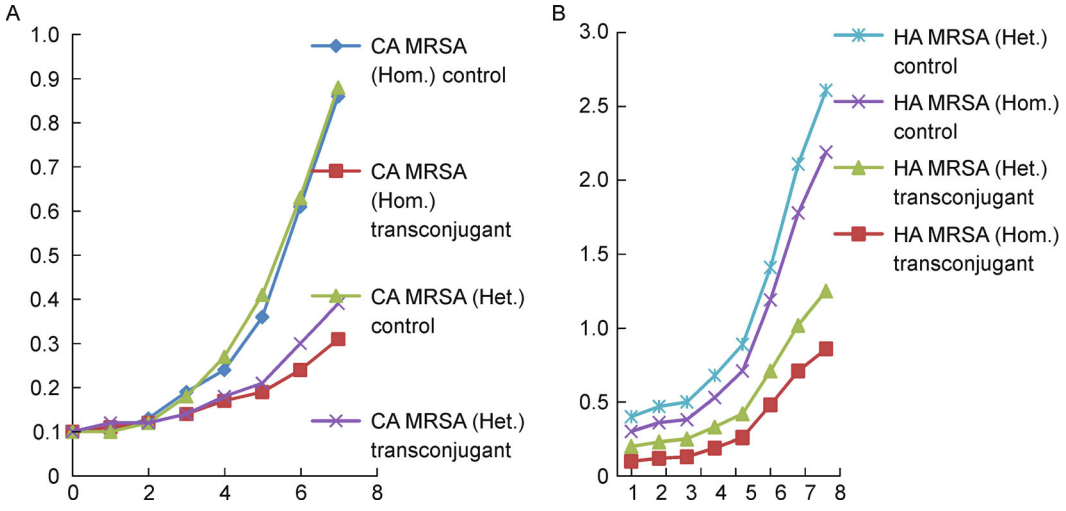

Figure 2 OD of transconjugants to determine growth kinetics after HGT.

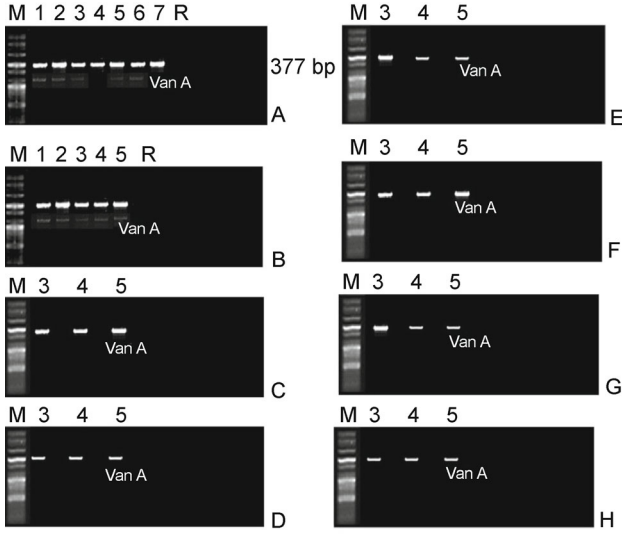

Figure 3 Gel electrophoresis of PCR products of donor, recipients and transconjugants. Detection of *Van A* gene by PCR using *Van A* primer set. PCR products were analyzed by 1% agarose gel electrophoresis. Reference strain "R" used was *Enterococcus faecium* ATCC 19434. M: 100bp DNA ladder marker; (A) (Lane 1,6,7; VRE (vancomycin resistant enterococci); (B) CA (hom.) MRSA transconjugant; (C) CA (het.) MRSA transconjugant; (D) HA (het.) MRSA transconjugant; (E) HA (hom.) MRSA transconjugant); Lane 2: Plasmid DNA of VRE for van A; Lane 3: CA (het.) MRSA transconjugants grown in broth with vancomycin; Lane 4: CA (het.) MRSA transconjugants grown in broth without vancomycin; Lane 5: CA (het.) MRSA transconjugants after many subcultures; (F): HA (het.) MRSA transconjugants grown in broth with vancomycin; (G) HA (het.) MRSA transconjugants after many subcultures; (H) HA (het.) MRSA transconjugants grown in broth without vancomycin.

required. Further, HA MRSA transconjugants showed a shortened lag phase followed by a relatively quick exponential phase in comparison with the CA MRSA transconjugants. HA strains are expected to face greater antibiotic pressure at hospitals, hence they may have adapted to take both the SCCmec III gene and the *Van A* gene at the same time with less of a fitness reduction. Biological cost is another factor responsible for the intra and interspecies transmission of resistance, and the global emergence of MRSA clones has been associated with their ability to compensate for the cost of harboring the SCC*mec* element to govern methicillin resistance (Ender et al., 2004). Community strains are now known to acquire the *Van A* resistance gene, probably because of unnecessary and sustained antibiotic pressure in the community. Earlier studies have established that deletion of the *mecA* gene in vancomycin intermediate-resistant *S. aureus* isolates suggests that co-resistance to beta-lactams and glycopeptides is unfavorable for *S. aureus* (Notoet al., 2008). It is of immense value to understand that despite this reduced fitness, CA MRSA are capable of acquiring, harboring, and maintaining the *Van A* gene in natural environments like river, ponds, and pools. It is an alarming situation, because HA MRSA with vancomycin resistant strains are shed into these environments or are drained into water bodies by patients' carelessness or the disorganized disposal of bandages in hospitals. To investigate the impact of river water on the growth dynamics of bacterial strains, basic physicochemical properties were assessed and showed pH 7.1, conductivity (u mho/cm) 150.81, total dissdved solids (mg/L) 86.59, TSS (mg/L) 125.91, total alkalinity (mg/L) 77.1, chemical oxygen demand (COD mg/L) 11.11, biological oxygen demand (BOD mg/L) 1.44, dissolved oxygen (mg/L) 8.96, and water temperature 11.1, which was adjusted to 30°C and 37°C.

In light of the evidence from this study, on the ability of vancomycin resistant MRSA to transfer and maintain resistance genes from hospital to community clones, and in laboratory conditions as well as in natural environmental conditions (in river water), calls for judicial and prudent use

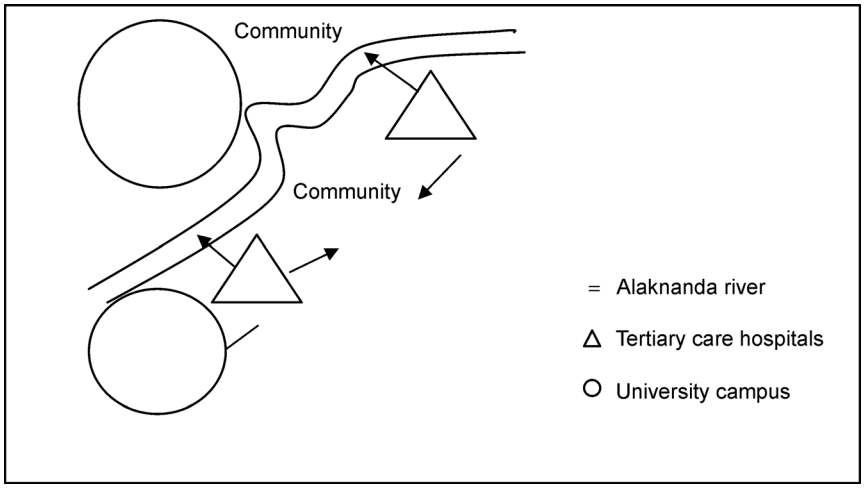

Figure 4 Line diagram of study area. Map not to be scaled.

of antibiotics as well as interventions in the dissemination of resistance genes in the community. Some habits like drug abuse and self-treatment without a prescription may have increased antibiotic pressure in the community. A more detailed epidemiological survey should be conducted in this region to determine whether these factors are associated with the dissemination of resistance. The study has local and global implications. It provides a platform to further investigate the reasons behind the acquisition of the *Van A* determinant by community associated MRSA. Clinicians, epidemiologists, and scientists should work together to monitor the inter and intraspecies spread of vancomycin resistance.

Conclusions

More study is required to understand the transfer of resistance determinants to the community. However, it may be concluded from the present study that methicillin resistance transfer from tertiary care centers to the community is not only a problem that needs to be taken care of, but that the resistance is transferring between different species level (from *E.faecalis* to *S. aureus*) and the capacity to retain the resistance in community MRSA is well above the average capacity. This suggests that CA MRSA strains are gaining the ability to harbor resistance determinants in natural environments. Hence, revised SOPs are required urgently for the disposal of hospital waste.

Acknowledgements

AK and ABB acknowledge the Ph.D. fellowship support provided by the University Grant Commission, New Delhi. AK thanks Preeti Ranawat (Ph.D. Scholar) and researchers at the laboratory of Microbiology, Department of Botany and Microbiology, Hemwati Nandan Bahuguna Garhwal University, for assistance with experiments and in developing the environmental model. Thanks are due to Miss Poonam Silori for critically reviewing the manuscript.

Compliance with ethics guidelines

None of the authors declare any conflicts.

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