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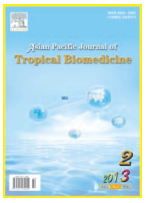

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A report on infection dynamics of inducible clindamycin resistance of *Staphylococcus aureus* isolated from a teaching hospital in India

Debasmita Dubey[1], Shakti Rath[1], Mahesh C. Sahu[1], Subhrajita Rout[1], Nagen K. Debata[1], Rabindra N. Padhy[2*]

[1]*Microbiology Department, IMS & Sum Hospital, Siksha 'O' Anusandhan University, Kalinga Nagar, Bhubaneswar 751003, Odisha, India*

[2]*Central Research Laboratory, IMS & Sum Hospital, Siksha 'O' Anusandhan University, Kalinga Nagar, Bhubaneswar 751003, Odisha, India*

PEER REVIEW

Peer reviewer

Dr. F. A. Olajubu, Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria.
Tel: +234802 393 1914
E-mail: olajubufa@yahoo.com

Comments

This is a good study in which the authors investigated the infection dynamics of inducible clindamycin resistance of *S. aureus* isolates from a hospital. The results are interesting. This study is significant for the hospital managers, decision makers, physicians and students to avoid cross infections in hospitals. It is also ringing alarm bells for the necessities in the solutions of nosocomial infection.

(Details on Page 152)

ABSTRACT

Objective: To investigate the infection of hospital- and community-acquired "erythromycin-induced clindamycin resistant" strains or D-test positives of clinical isolates of *Staphylococcus aureus* (*S. aureus*) (with and without methicillin resistance) in a hospital. **Methods:** Strains of *S. aureus* isolated from clinical specimens were subjected to D-test and antibiotic profiling. **Results:** Of the total 278 isolates, 140 (50.35%) were D-test positives and the rest were D-test negatives. Further, of 140 (100%) positives, 87 (62.14%) and 53 (37.85%) strains were from males and females, respectively. Of 140 (100%) positives, 117 (83.57%) were methicillin resistant *S. aureus* and 23 (16.42%) were methicillin sensitive *S. aureus*; of 140 strains, 103 (73.57%) strains from persons with and 37 (26.42%) were without related infections; of 140 strains, 91 (65%) and 49 (35%) were from hospital- and community-acquired samples, respectively. In 140 strains, 118 (84.28%) with comorbidities and 22 (15.71%) without comorbidities cases were recorded; similarly, persons with prior antibiotic uses contributed 108 (77.14%) and without 32 (22.85%) positive strains. These binary data of surveillance were analyzed by a univariate analysis. It was evident that the prior antibiotic uses and comorbidities due to other ailments were the determinative factors in D-test positivity, corroborated by low *P* values, $P=0.0011$ and 0.0024, respectively. All isolates (278) were resistant to 17 antibiotics of nine groups, in varying degrees; the minimum of 28% resistance for vancomycin and the maximum of 97% resistance for gentamicin were recorded. Further, of 278 strains, only 42 (15.1%) strains were resistant constitutively to both antibiotics, erythromycin resistant and clindamycin resistant, while 45 (16.2%) strains were constitutively sensitive to both antibiotics (erythromycin sensitive and clindamycin sensitive). Further, of the rest 191 (68.7%) strains were with erythromycin resistant and clindamycin resistant, of which only 140 (50.35%) strains were D-test positives, while the rest 51 (18.34%) strains were D-test negatives. **Conclusions:** In view of high prevalence of D-test positive *S. aureus* strains, and equally high prevalence of multidrug resistant strains both in community and hospital sectors, undertaking of D-test may be routinely conducted for suppurative infections.

KEYWORDS

Antibiotics, Community-acquired, D-test, Erythromycin resistance, Hospital-acquired, Inducible clindamycin resistance, MRSA, MSSA, *Staphylococcus aureus*

1. Introduction

As a commensal, *Staphylococcus aureus* (*S. aureus*) colonizes asymptomatically nares of nose, skin and soft tissues of healthy individuals. But through bloodstream infection, a number of casual to serious ailments are caused, such as skin reactions, rhinitis, otitis media infection, mastitis, suppurative wounds, osteomyelitis, septic arthritis, urinary tract infections, and several life-threatening invasions, *i.e.* pneumonia, septicemia, bacteraemia, endocarditis and toxic-shock syndrome[1]; an infectious *S. aureus* brings a retinue of damnedest comorbidities, even fatality. *Per se*, *S. aureus* has been the most prevalent Gram-positive pathogen in India[2]. For the *in vivo* control,

*Corresponding author: Dr. Rabindra N Padhy, CSIR Scientist, Central Research Laboratory, IMS & Sum Hospital, Siksha 'O' Anusandhan University, K-8, Kalinga Nagar, Bhubaneswar 751003, Odisha, India.
Tel: +91 674-2432034
E-mail: rnpadhy54@yahoo.com

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erythromycin has been in use since 3-4 decades and resistance to it by *S. aureus* has been reported since long[3]. Its invasive/insinuative nature is evident with its aggrandizement of resistance to multiple drugs, including vancomycin. And, methicillin resistant *S. aureus* (MRSA) was also found resistant to other preferred antibiotic, streptogramin B. Consequently, clindamycin, another wonted drug against Gram-positive pathogens was in use for *S. aureus*.

Surprisingly, inducible clindamycin resistance (Cd-r) of both methicillin sensitive *S. aureus* (MSSA) and MRSA, due to erythromycin resistance (Er-r) had been accentuated[4]. It was ascertained that, Cd-r mutants harbor the *erm* gene [Er-r gene that induces resistance to the macrolides, lincosamides and streptogramin B (MLSB) group, by a methylation at the 23s r-RNA subunit that leads to methylation,5]. There are two types of Er-r *S. aureus* strains, *i.e.*, with and without the MLSB gene. In the presence of erythromycin, the strain with the MLSB gene induces resistance to clindamycin in the "Er-r, Cd-s" strain, conferring clindamycin resistance to the original Cd-s strain, eventually causing the well-known flattening of the clindamycin inhibition zone towards the erythromycin disc, so that the shape "D" is seen in the clindamycin zone or "D-test positivity" (Figure 1). Since, failure in the therapy with clindamycin used against *S. aureus* had been frequently met[4,6], the D-test procedure is often recommended for checking the efficacy of the empiric use of clindamycin against isolated staphylococci in most hospitals to avoid the unbeknown pervasive error in the therapy, due to MLSB resistance. Admittedly, it is the standard procedure, being simple for checking the inducible *erm* mediated MLSB resistance in MRSA and other staphylococci. Moreover, inducible-MLSB *S. aureus* strains have been isolated independently with resistant patterns for a number of antibiotics in use, in diverse geographical zones[3]; their abundance have been reported up to the saturnine height of 94% of *S. aureus* isolates, a decade ago[7].

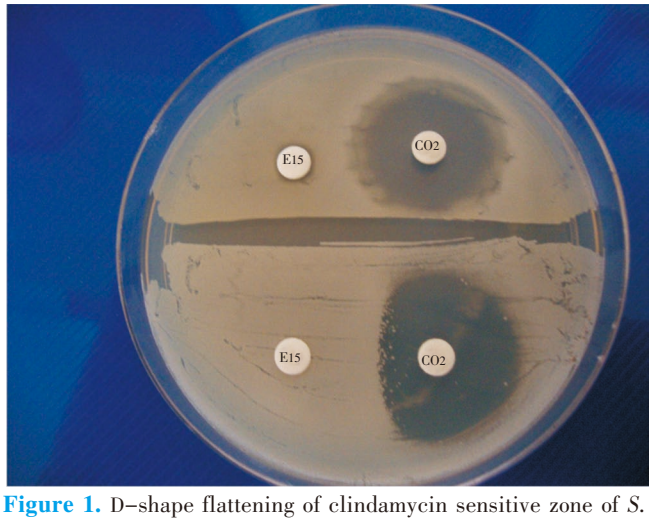

Figure 1. D-shape flattening of clindamycin sensitive zone of *S. aureus* induced by erythromycin resistance.
Both bacterial strains plated were erythromycin resistant, but the strain with MLSB gene had D-shape flattening or D-test positivity, while the other strain without MLSB gene had no D-shape zone from clindamycin toward the erythromycin disc.

This fixated study characterizes the prevalence of *S. aureus* in samples from in-house patients, hospitalized in wards, cabins, intensive care units, and neonatal intensive care units for 2 or more d, taken as hospital-acquired (HA), and samples from patients who regularly/intermittently visit outpatient department, taken as community-acquired (CA). Further, since clindamycin is frequently used empirically before results of cultures of clinical samples could be obtained for patients with aerobic-anaerobic infection from intra-abdominal sepsis, aspiration pneumonia, soft tissue infections, cellulites and post-surgical wounds, *etc.*[8], it was a deliberate attempt of surveillance in probing to the occurrence of D-test positive *S. aureus* strains, in a resource-limited setting. Obviously, a post-hoc analysis on the cause of failure in to-do-away-with the multidrug resistant strains of this pathogen by an empiric treatment with any member of the MLSB group, specifically the clindamycin would be a clinical misdemeanour. A heedful univariate analysis of the bivalence of D-test results with several hospital factors such as, sex, presence of comorbidities, *etc.*, vindicates this study. Further, an antibiogram of a spectrum of 278 isolates of *S. aureus* with 17 antibiotics was obtained that gave an idea on the prevalence of the insidious infection-dynamics and the associated shenanigans of this notorious super-bug of health domain, for a benefit of apothecary in dove-tailing suitable drugs and to decrease unwarranted increases in the growing cost of hospital care, in face of the intimidating erythromycin-induced MLSB resistance.

2. Materials and methods

2.1. Isolation and antibiotic susceptibility

The study was conducted for a period of 6 months (April to September 2011) and a total of 278 strains of *S. aureus* were isolated from different clinical samples from HA and CA sources of Institute of Medical Science & Sum Hospital. Isolated strains were identified by using the standard microbiological procedures[9]. The MSSA strain, Microbial Type Culture Collections strain number 7443 was used as the reference control. This strain and all isolated strains were subjected to antibiotic sensitivity test, by the disc diffusion method, detailed previously[10].

2.2. Detection of MRSA

For the cefoxitin disc diffusion test, a 0.5 McFarland standard equivalent suspension of a test isolate was plated for lawn culture on a Muller-Hinton agar plate; a cefoxitin disc 30 μg/disc was placed on the lawn-center. Plates were incubated at 37 °C for 18 h and inhibition-zone diameters were measured; a value ⩾19 mm was recorded as methicillin resistant and a value, ⩽20 mm was considered as methicillin sensitive[11]. For the chromogenic agar media test, pure clinical isolates of *S. aureus* were streaked onto MRSA-agar, the Hichrome-MeReSa agar (HiMedia, Mumbai), and were incubated for 24 h at 37 °C; MRSA strains had blue colonies and MSSA strains had white colonies[12].

2.3. D-test

Isolates that were "Er-r, Cd-s" were tested for inducible Cd-r, by susceptibility to clindamycin 2 μg/disc and erythromycin 15 μg/disc levels along with the reference strain, according to CLSI criteria[13]. Erythromycin and clindamycin discs (HiMedia, Mumbai) were placed (17±2) mm apart (edge to edge) on a Muller-Hinton agar plate, incubated at 37 °C for 18 h and D-test positivity was identified by the flattening of clindamycin zone between erythromycin and clindamycin discs. Any isolate with "Er-r, Cd-r" was considered as constitutive MLSB resistant strain[14].

3. Results

A total of 278 strains of *S. aureus* were isolated from clinical samples, pus, different swabs, urine, body fluids and blood, in the cited order of prevalence, both in HA and CA sources (Table 1). Of 278 (100%) strains, 152 (54.67%) isolates were from HA and 126 (45.32%) from CA samples. Of 152 HA isolates, 129 (46.4%) strains were MRSA and 23 (8.27%) were MSSA, whereas of the total 126 (45.32%) CA strains, 97 (34.89%) were MRSA and 29 (10.43%) were MSSA (Table 1 and Figure 2).

Table 1

Occurrence of inducible clindamycin resistant isolates as D-test positives in total *S. aureus* strains in different clinical samples.

Source samples	Hospital acquired		Community acquired	
	MRSA	MSSA	MRSA	MSSA
Pus	41 (32)	6 (4)	33 (14)	9 (3)
Wound swabs	32 (18)	4 (2)	24 (7)	5 (3)
Skin swabs	21 (13)	4 (2)	17 (8)	4 (2)
Nasal swabs	16 (7)	3 (2)	12 (4)	4 (2)
Urine	9 (4)	3 (2)	5 (3)	3 (-)
Body fluids	7 (4)	2 (-)	3 (2)	3 (-)
Blood	3 (-)	1 (-)	3 (1)	1 (1)
Total	129 (78)	23 (12)	97 (39)	29 (11)

Numbers in parenthesis represents D-test positives strains, the total being 140, out of the grand total number of 278 strains.

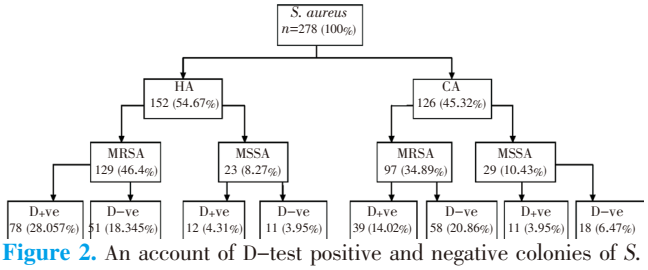

Figure 2. An account of D-test positive and negative colonies of *S. aureus* with respect to other variables.

Of the total 278 strains, 140 (50.35%) were D-test positive and 138 (49.64%) were D-test negative. D-test positivity/negativity had concern to other variable factors, sensitivity to methicillin, source of strains, sex, other associated ailments, other infections, and prior antibiotic use. Thus, the following bivalents with respect to both D-test positive and negative strains were monitored (Table 2): 1. MRSA/MSSA; 2. HA/CA sources; 3. Males/females; 4. Presence/absence of associated comorbidities (presence/absence of diabetes-related ailments any other problems like cardiac complaints, noteworthy diseases, *etc.*); 5. Presence/absence of related infections; and 6. Their prior antibiotic uses within >90 d (Table 2). The *P*-value for two pairs of data, D-test positive/negative for MRSA/MSSA pair was 0.3242, which signified that there was no statistically respectable difference of D-test positivity between MRSA/MSSA pair (for 140 strains of D-test positives and 138 negatives); similarly, the D-test positive/negative cases related to sex were not significant at the level. On the contrary, for the rest 4 pairs of bivalents as mentioned above (numbers 2, 4, 5 and 6), *P*-values were statistically significant, signifying there were statistically respectable differences (Table 2). Further, for both the bivalent data for "prior antibiotic use", and "presence or absence of comorbidities" with *P*=0.0011 and 0.0024, respectively, for D-test positive/negative cases confirm the difference statistically. In other words, the distribution of MLSB gene in samples from patients "with prior antibiotic use" and "presence of comorbidities" had determinative roles in D-test positivity.

Table 2

Univariate analysis of D-test positive and D-test negative isolates of *S. aureus**.

Variables		D-test positive	D-test negative	*P*-value	Odds ratio	Range (%95 CI)
Strains	MRSA	117	109	0.3242	1.3534	0.7381-2.4816
	MSSA	23	29			
Sources	HA	91	67	0.0050	1.9680	1.2157-3.1859
	CA	49	71			
Sex	Male	87	102	0.0330	0.7403	0.4546-1.2056
	Female	53	46			
Comorbidity	Present	118	96	0.0024	2.3466	1.3113-4.1993
	Absent	22	42			
Related infections	Present	103	116	0.0281	1.0070	0.6019-1.6877
	Absent	37	42			
use >90 d Prior antibiotic	Present	108	82	0.0011	3.5396	2.153-5.8191
	Absent	32	86			

*See text for detailed information on variables and for abbreviations.

The univariate analysis of surveillance data revealed that MRSA detection had 1.3534 times more risk factor or vulnerability to express the MLSB gene along with the acquiring of Er-r factor than MSSA prevalence in causing D-test positivity. Similarly, there was 1.9680 times more chance of prevalence of the MLSB gene in Er-r *S. aureus* from HA samples to have often D-test positivity than those from CA samples. Patients with other comorbidities had been recorded to have 2.3466 more chance than patients without any comorbidity for positivity. And patients with a history of prior antibiotic uses had the highest value of 3.3596 more chance than patients without any such history for positivity. On the other hand, males were found to have 0.7403 times less chance in acquiring inducible Cd-r than females, in this surveillance. However, patients with or without other related infections had an equal chance of acquiring inducible Cd-r (Table 2).

Resistance at a minimum of 36% and 13% for daptomycin, 34% and 28% for vancomycin, and the maximum of 97% and 95% for gentamicin and 95% and 86% for oxacillin were recorded for HA and CA *S. aureus* isolates, respectively. Further, it was clear that resistant values of isolates to erythromycin were 83% and 67%, and those were independently resistant to clindamycin by 76% and 81% at CA and HA isolates, respectively (Table 3).

Table 3

Percentage of resistance of *S. aureus* to 17 antibiotics of various groups with both hospital acquired and community acquired strains (*n*=278).

Antibiotic group	Antibiotics (μg/disc)	HA isolates (%)	CA isolates (%)
Aminoglycosides	Amikacin 30	89	74
	Gentamicin 10	97	95
β-lactams	Amoxyclav 30	85	76
	Ampicillin 10	88	68
	Oxacillin 1	95	86
	Penicillin 10	36	56
Fluoroquinolone	Gatifloxacin 05	78	67
Glycopeptides	Teicoplanin 10	80	59
	Vancomycin 30	28	34
Lincosamide	Clindamycin 2	81	76
Lipopeptide	Daptomycin 30	36	13
Macrolides	Azithromycin 15	72	54
	Erythromycin 15	83	67
Sulfonamide	Co-trimoxazole 5	78	49
Stand-alone antibiotics	Chloramphenicol 30	72	61
	Linezolid 30	67	37
	Tetracycline 30	45	34

These multidrug resistant isolates were at such a high abundance that antibiotics, gentamicin and gatifloxacin were excluded deliberately for *S. aureus*, from antimicrobial stewardship programme.

On separate lawn cultures of all isolates, two discs-erythromycin 15 μg/disc and clindamycin 2 μg/disc were used for checking the susceptibility pattern. Of 278 (100%) strains, 42 (15.1%) were constitutively resistant to both antibiotics, while 45 (16.1%) more strains were constitutively sensitive to both. The rest 191 (68.7%) strains were expected to be D-test positive, but only 140 (50.35%) strains had positivity, while the rest 51 (18.34%) strains were negatives (Table 4). Thus, this study had data of constitutive resistant pattern for both (Cd-r, Er-r) and constitutive susceptibility pattern for both (Er-s, Cd-s). These phenotypes in the routine isolation procedure were isolated, which helped to assess the prevalence of D-test positives among MSSA and MRSA isolates. It was found that the double constitutive resistance of MSSA was totally absent in both HA and CA samples; on the other hand, double constitutive sensitive phenotype (Er-s, Cd-s) were 4 and 8 isolates in total from MSSA isolates, whereas this phenotype occurred as 7 and 26 from MRSA isolates, in HA and CA cohorts, respectively (of total D-test positives). This clearly indicated that negligible fractions of both constitutive sensitive and resistant phenotypes were prevalent, which were unsuitable for checking D-test positivity (Table 4).

Table 4

Patterns of sensitivity and resistance to antibiotics in strains of *S. aureus* to erythromycin and clindamycin during D-test.

Strains	Hospital acquired		Community acquired		Total
	MRSA	MSSA	MRSA	MSSA	
Er-r + Cd-r Constitutive resistant	25	0	17	0	42
Er-s + Cd-sV Constitutive sensitive	7	4	26	8	45
Er-r + Cd-s D-test negative	18	7	21	5	51
Er-r + Cd-s D-test positive	78	12	39	11	140

Er-r: erythromycin resistant; Er-s: erythromycin sensitive; Cd-r: clindamycin resistant; Cd-s: clindamycin sensitive. Both Er-r and Cd-r strains were taken as of constitutive resistance; both Er-s and Cd-s strains were taken as of constitutive sensitive. Total number of D-test positives=140; total number of *S. aureus* strains=278.

4. Discussion

During the unifying assessment of 278 strains resistant to 17 antibiotics, the infection-dynamics of this iconic notorious pathogen was discernible with the minimum of 28% resistance to vancomycin and the maximum of 97% resistance to gentamicin. Indeed, occurrence of high percentage of resistance to daptomycin at 36% in HA samples is of high clinical concern in this study. The most striking situation was that *S. aureus* strains have emerged with concomitant resistance to many commonly used antibiotics of groups seen here, also as seen elsewhere[1]. Surprisingly, the imperiling value of ~30% epidemiological prevalence of vancomycin resistant *S. aureus* in this hospital is a matter of concern; these could be due to errors in manual method of determining antibiotic susceptibility pattern in a resource limited settings with the absence of an automated technique, the use of vancomycin in empiric therapy and overall, the absence of a stringent antibiotic policy in local hospitals, to state contemplatively. Moreover, in a European country, of 750 clinically isolated *S. aureus* strains, 38% D-test positives were obtained in CA and 67% in HA-MRSA isolates; but the D-test positive figure for HA-MSSA was 63.6%; further, MRSA isolates were often found resistant to cephalosporins, cefems and other β-lactams, ampicillin-sulbactam, amoxyclav, ticarcillin-clavulanic acid, piperacillin-tazobactam and the carbapenem, imipenem[15]. According to our survey, the percentage of D-test positivity in CA isolates was lower than that of HA strains.

Strains that were Er-r when plated with Cd-s were expected to have D-test positivity, but out of the total 191 (Er-r, Cd-s) isolates, only 140 strains were D-test positive. Thus, a cohort of 51 Er-r strains was unable to induce D-like flattening of clindamycin-inhibition zone, due to the absence of MLSB gene. From the analysis of D-test positivity with variable factors, MRSA/MSSA, sex, absence/presence of comorbidities, *etc.*, it was evident that the distribution pattern of MLSB gene was not universal among all Er-r isolates that could be the cause of 140 D-test positives only, among 191 Er-r strains. It is imperative that some other mechanism is also involved in Er-r, at least with 51 stains herein that could be the active efflux mechanisms to evade antibiotics of the MLSB group by an intrinsic gene[5]. Moreover, in this study the two types of phenotypes, D and D+, basing on the size of the clear zone around the erythromycin disc less than 6 mm for former and more than 8 mm for the later as described[16], were not detected in this study.

Among 244 clinical isolates of staphylococci reported from Karnataka, India, 13.1% strains had inducible clindamycin resistance with the MLSB phenotype; among them, 10 isolates were MRSA (38.4% of the total MRSA), 16 were MSSA (12.9% of the total MSSA) and 6 were "coagulase-negative staphylococci" or CONS, *i.e.*, 6.3% of the total isolated CONS[17]. In another laboratory from Karnataka, 10% isolates had inducible clindamycin resistance, 9% had constitutive resistance and 8% had MS phenotype. Inducible resistance and constitutive resistance were found to be higher in MRSA as compared to MSSA (20%, 16% and 6%, 6%, respectively)[18]. The prevalence of MLSB strains both in CA and HA *S. aureus* isolates, as well as the prevalence of CA-MRSA strains were identified as clinical predictors of both CA-MRSA and MLSB, in Albama, USA[3]. Among 402 *S. aureus* isolates, the prevalence of MLSB was 52%, of which 50% of MRSA and 60% of MSSA isolates were MLSB; CA-MRSA were 14% of all isolates and had a lower prevalence of MLSB than HA-MRSA: 33% versus 55%, respectively[3]. A total of 159 staphylococcal isolates from burn patients in the Tripoli Burn Center were tested for inducible clindamycin resistance, which was detected in 66.2% of 65 MRSA isolates and in none of 55 MSSA, 10 methicillin-resistant CONS and 29 methiclin-sensitive CONS isolates[19]. It was reported that 88.6% MRSA isolates were Er-r and 52.3% were Cd-r in Iran; values of resistance in MSSA strains to erythromycin and clindamycin were 22% and 11.4%, respectively. Inducible

clindamycin resistance was detected in 20.5% MRSA isolates; but, 52.3% of MRSA isolates and 7.3% of MSSA had constitutive MLSB phenotype[20].

The round zones due to erythromycin and clindamycin radiating out from each disc partially were observed. Erythromycin molecules reach the outer region of clindamycin zone prior to clindamycin molecules. The presence of the MLSB genotype in the lawn of Er-r stain led a methylase translation permitting the growth in this region, despite the diffusion of an inhibitory concentration of clindamycin. The D-test positives render clinical difficulty, due to the failure of clindamycin treatment of MRSA; eventually the D-test becomes an implicit trust. *In vitro* testing of isolates with MLSB genotype demonstrates clindamycin susceptibility. But macrolide inducible DNA sequence that preceded the *erm* (methylase) open reading undergoes mutation, substitution or deletion that generate a readily translatable (now the constitutive MLSB) stretch of DNA; its secondary m-RNA structure was recorded to be about of 1-2 million base pairs[21].

In the realm of imagination, origin of multidrug resistance in *S. aureus* has many possibilities: 1. The development of exquisite clonal nexuses of *S. aureus* is fast due to the genome simplicity[21]. 2. "Positive selection pressure", the accepted/viable concept of evolution could be valid, not least because of the availability of antibiotics and their degraded toxic products readily in nature, such as in untreated hospital and community drains, but an altered influx potential in disallowing an antibiotic through plasma membrane, could often be the mechanism involved for resistance, as exemplified elsewhere[22]. 3. Genetic recombination mechanisms-conjugation and transformation should occur more readily than expected in untreated hospital sewage system, because all sorts of bacteria with grading levels of antibiotic resistance are physically together, and DNA from lysed cells would be readily available for uptake by living cells, trickling genome improvement as discussed[10]. In developing sections, the scientific disposition of hospital waste should be expected to be in a developing state, unwittingly-giving space for pathogen spreads. 4. Horizontal transfer/suffusion of drug-resistant pathogens to both community and hospital settings is expected because of the accumulated grime from crowding of patients and their attendants in resource limited hospital settings; a priory, slum areas of developing zones of developing countries might be conducive to pathogen spread in community. When, a bacterial strain musters a set of drug-resistant characters as an armamentarium against antibiotics of present time in an individual patient, it acts as a doppelgänger during improvement in all strains of the species in the patient-body, as if with a snowball decent time-to-time. Thus, spread of the novel strain in community is the aftermath. Such events occur continually and independently with each pathogen. This is the mechanism of transformation of the harmless commensal *S. aureus* to the ghoulish, intractable, perilous and wily superbug MRSA[23].

Binary outcomes of surveillance data elucidated that "prior antibiotic uses" and "comorbidities due to other ailments" were determinative factors of D-test positivity. It could be identified here that inducible MLSB strain was widespread in hospital sectors, so D-test protocol need to be included as the routine diagnostic procedure for suppurative ailments of incoming patients. But, in the community sector, there was less Cd-r *S. aureus* strains. This study indicated negligible fractions of constitutive "sensitive" and "resistant" phenotypes that were pejorative for D-test positivity. In view of the high prevalence of D-test positive *S. aureus* strains both in community and hospital sectors, undertaking of D-test may be routinely conducted.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

An infectious *S. aureus* brings a chain of comorbidities, even fatality, and *S. aureus* has been the most prevalent Gram-positive pathogen. For the *in vivo* control, erythromycin has been in use since 3-4 decades and resistance to it by *S. aureus* had been reported since long. Its invasive nature is evident with its attainment of resistance to multiple drugs, including vancomycin. And, methicillin resistant *S. aureus* (MRSA) was too found resistant to the other preferred antibiotic, streptogramin B. Consequently, clindamycin, another mostly used drug against Gram-positive pathogens was in use for *S. aureus*. Surprisingly, inducible clindamycin resistance (Cd-r) of both methicillin sensitive *S. aureus* (MSSA) and MRSA, due to erythromycin resistance (Er-r) had been known. It was ascertained that, Cd-r mutants harbor the *erm* gene [Er-r gene that induces resistance to the MLSB group (macrolides, lincosamides and streptogramin B), by a methylation at the 23s r-RNA subunit that leads to methylation. In the presence of erythromycin, the strain with the MLSB gene induces resistance to clindamycin in the "Er-r, Cd-s" strain, conferring clindamycin resistance to the original Cd-s strain, eventually causing the well-known flattening of the clindamycin inhibition zone towards the erythromycin disc, so that the shape "D" is seen in the clindamycin zone or "D-test positivity".

Research frontiers

Since, failure in the therapy with clindamycin used against *S. aureus* had been frequently met, the D-test procedure is often recommended for checking the efficacy of the empiric use of clindamycin against isolated staphylococci in most hospitals to avoid the unbeknown pervasive error in the therapy, due to MLSB resistance.

Related reports

Inducible-MLSB *S. aureus* strains have been isolated independently with resistant patterns for a number of antibiotics in use, in diverse geographical zones; their abundance have been reported up to the height of 94% of *S. aureus* isolates (Patel *et al*. 2006; Sibbery *et al*. 2002; Jorgensen *et al*. 2004).

Innovations and breakthroughs

It could be identified in this study that inducible MLSB strain was widespread in hospital sectors, so D-test protocol need be included as the routine diagnostic procedure for suppurative ailments of incoming patients. But, in community sector, there was less Cd-r *S. aureus* strains. This study indicated negligible fractions of constitutive "sensitive" and "resistant" phenotypes that were pejorative for D-test positivity. Indeed, occurrence of high percentage of resistance for daptomycin at 36% in hospital aquired samples are of high clinical concern, in this study. The most striking situation was that *S. aureus* strains have emerged with concomitant resistance to many commonly used antibiotics seen here.

Applications

In view of high prevalence of D-test positive *S. aureus* strains both in community and hospital sectors, undertaking of D-test may be routinely conducted to prevent mis-matches out of empiric use of clindamycin against this super-bug of health domain, for wound sites.

Peer review

This is a good study in which the authors investigated the infection dynamics of *S. aureus* isolates from a hospital. The results are interesting. This study is significant for the hospital managers, decision makers, physicians and students to avoid cross infections in hospitals. It is also ringing alarm bells for the necessities in the solutions of nosocomial infection.

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