

CHARACTERISTICS AND ANTIBIOGRAM OF SMALL COLONY VARIANTS OF *Staphylococcus aureus* FROM A TERTIARY CARE HOSPITAL IN EASTERN INDIA

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ABSTRACT

Introduction: Small colony variants (SCVs) are morphotypes of *Staphylococcus aureus* that are about one-tenth the size of wild-type *Staphylococcus aureus*. They are slow growing and produce indolent infections, and are usually auxotrophic for Vitamin K, Carbon dioxide or hemin. They are prone to be missed unless meticulous suspicion is there, because coagulase test is often delayed and hemolysis on blood agar can be negative. They are also sometimes resistant to multiple antibiotics. These atypical variants hence need to be studied further in these respects. **Materials and methods:** We here present the microbial and clinical characteristics of these variants isolated in our laboratory by routine culture methods and biochemical tests and describe their in-vitro antibiotic susceptibilities. **Results:** Different type of SCVs auxotrophic for various substrates were isolated during this period. **Conclusion:** SCV *S. aureus* can often be missed and need to be identified and categorised properly for empirical treatment.

KEYWORDS: Small Colony Variants, Auxotrophic, Antibiogram

Staphylococcus aureus can cause a variety of infections like skin and subcutaneous infections, pneumonia, osteomyelitis, toxic shock syndrome and infective endocarditis among other infections. (Bhattacharyya S *et al*, 2012) They sometimes produce a phenotypic variant called small colony variant that can cause persistent infections due to their ability to persist intracellularly (Bhattacharyya S *et al*, 2014). They are often auxotrophic for Vitamin K (menadione), Carbon dioxide, Thymidine or hemin (Al Laham, 2013). SCVs are also found in *Staphylococcus epidermidis*, although they were initially described in *Salmonella* Typhi, way back in 1910 by Jacobsen (Al Laham, 2013). They are also atypical in having reduced alpha-toxin gene expression, hence causing less damage to host cells (Von Eiff, 2000). Occurrence of SCV *S. aureus* is found to be especially common in chronic diseases like cystic fibrosis and osteomyelitis (Von Eiff, 2000). We here describe our own experience in isolating SCV *Staphylococcus aureus* from various clinical samples over 11-month period, in a tertiary care hospital in Eastern part of India.

MATERIALS AND METHODS

This was a laboratory based observational study, carried out in the Department of Microbiology of All India Institute of Medical Sciences, Patna, from February 2014 to January 2015. *Staphylococcus aureus* strains were isolated from different clinical samples routinely received in the laboratory from outpatient and inpatient departments. Samples were first inoculated on 5% Sheep blood agar and Mac Conkey agar, in case of

samples like pus, catheter tip, turbid blood culture bottles and wound discharge. *Staphylococcus aureus* was identified by observing Gram positive cocci arranged in clusters, catalase positivity and Oxidase negativity, along with positive reaction for slide coagulase (using pooled human plasma) and Mannitol fermentation using 1% Mannitol (w/v) in peptone water with Andrade's indicator, along with golden yellow pigment if present.

Following identification, antibiotic susceptibility of the isolates was performed by Kirby-Bauer's disk diffusion technique as per CLSI protocol (Clinical Laboratory Standards Institute, 2006). The following antibiotic disks were used: Cefoxitin (30 µg), Levofloxacin (5 µg), Erythromycin (30 µg), Amikacin (30 µg), Clindamycin (2 µg) and Netilmicin (10 µg) (HiMedia Labs, New Delhi, India).

Small colony variants were identified by small (about one-tenth diameter) size of colonies on solid media described before. Auxotrophisms were checked by growing the colonies in an atmosphere of increased CO₂ availability (CO₂ incubator), chocolate agar (hemin), and Vitamin K (menadione, 15 µg) disks. Auxotrophism was defined as reversion to wild type colony (about 1.5-2 mm diameter) after adding that particular factor, i.e. if colonies reverted to wild type by growing in CO₂-rich atmosphere, they were defined as CO₂ auxotrophic.

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RESULTS

Out of a total of 71 different isolates of *Staphylococcus aureus* isolated and identified in the laboratory during this 11-month period, 11 were found to be Small colony variants (15.5%). Out of these 11 SCV isolates, 5 were recovered from pus samples, 2 each from urine samples and wound discharge, and 1 each from sputum (respiratory tract) and central venous catheter tip. Out of these 11 patients, 9 were female subjects and only 2 were male. Thus a gross female preponderance was observed.

Mean age of the subjects having SCV *S. aureus* infection was 25.9 years. Thus infection by SCV was found most commonly in young people.

In 2 isolates, SCVs were found to grow along with wild-type *S. aureus* (mixture). In remaining 9 isolates, pure growth of SCV *S. aureus* was found. In the respiratory isolate, SCV *S. aureus* was found to grow along with *Moraxella catarrhalis*, another respiratory pathogen.

Biochemical Characteristics: All these isolates were coagulase positive, but 9 out of these 11 isolates (81.8%) were delayed coagulase positive, and that too, after adding excess plasma. All were Mannitol non-fermenters and catalase positive.

Auxotrophism: Only 4 SCV isolates (36.4%) were auxotrophic for Carbon-dioxide. No Vitamin-K auxotroph was found. Out of the remaining 7, 3 were Thymidine auxotroph and the rest 4 showed indeterminate auxotrophism.

Susceptibility: Only 2 isolates (18.18%) were Cefoxitin resistant, correlating with Methicillin resistance. The corresponding figures for Amikacin, Levofloxacin, Erythromycin, Netilmicin, Cotrimoxazole and Clindamycin were 0%, 9.09%, 27.27%, 9.09%, 27.3% and 18.18%, respectively.

DISCUSSION

Small colony variants of *Staphylococcus aureus* are sometimes found in antibiotic-refractory infections like osteomyelitis, chronic airway infections in cystic fibrosis and device-related infections (Garcia *et al*, 2013). They are often resistant to beta-lactam antibiotics due to an exceptionally slow rate of multiplication, and aminoglycoside antibiotics due to auxotrophicity for Vitamin K and Hemin, which are key components of the electron transport chain(Garcia

et al, 2013). Our study shows no resistance to amikacin, an aminoglycoside antimicrobial, possibly due to the fact that we isolated CO₂ auxotrophs and Thymidine auxotrophs predominantly from all SCV isolates. Environmental pressure such as administration of antibiotics, have been proved to select for SCV cells that are frequently found coexisting with their parent wild-type strains in a mixed bacterial culture(Melter and Rajodevic, 2010). Their altered metabolism or auxotrophism can result in quite long generation time (about one-sixth to one-ninth that of wild-type *S. aureus*) and thus small colony phenotype on solid media(Melter and Rajodevic, 2010).In some studies, Thymidine auxotrophic SCV s of *S. aureus* have been found to be most common in cystic fibrosis patients(Yagci *et al*, 2013). Carbon dioxide auxotroph SCVs are rare among SCVs of *S. aureus*, are very frequently Methicillin resistant (about 60-65%) (Gomez-Gonzalez *et al*, 2010). Although the general prevalence of SCVs of *S. aureus* in all clinical specimens in a microbiology laboratory has been estimated to be approximately 1%, SCVs are recovered more frequently from certain predisposed patients such as those with cystic fibrosis (Gomez-Gonzalez *et al*, 2010). CO₂ auxotroph SCVs of *S. aureus* are often found in conjunction with wild type *Staphylococcus aureus*, according to some studies, and can sometimes even fail to grow aerobically (Gomez-Gonzalez *et al*, 2010). In case of our study, the frequency of isolation of SCV from all *S. aureus* isolates was about 15%, which is quite high; this may be due to inappropriate and overuse of antibiotics, at least in our area, since most patients came from nearby areas. Another interesting fact is the significantly more isolation of SCVs from female patients compared to males, which can partly be due to more health care seeking behaviour in male subjects, resulting in less or improper treatment and persistence of the pathogen in the host cells (Yamasaki-Nakagawa *et al*, 2001). This study highlights that SCVs of *S. aureus* should be looked for and identified wherever possible, since they cause a plethora of infections (Gomez-Gonzalez *et al*, 2010). SCVs can also be often refractory to multiple antibiotics and studying and carrying out antibiogram is thus important.

CONCLUSIONS

SCVs of *S. aureus* can often confuse a laboratory physician and lead to misidentification. They need to be looked into and identified meticulously from clinical samples and reported. They also need to be categorised properly for empirical treatment.

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