

Study of Prevalence, AntibioGram and Virulence Determinants of Small Colony Variants of Different Bacteria Isolated in a Tertiary Care Hospital

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ABSTRACT

Introduction: Small colony variants are slow growing subpopulations of bacteria. They are often resistant to multiple antibiotics. It is important to know the prevalence and susceptibility pattern of these bacteria in order to institute correct empirical therapy.

Materials and Methods: We studied these subpopulations of bacteria in various samples and also their virulence factors and susceptibility pattern by conventional methods, since if high degree of antibiotic refractoriness is noted, anti-virulence strategies can be considered.

Results: *S. aureus* and *E. coli* were the commonest bacteria showing SCV phenotype, and protease and lipase were the commonest virulence traits found in *S. aureus* SCV and *E. coli* SCV, respectively.

Keywords: SCV, Susceptibility, Virulence.


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INTRODUCTION

Small colony variants (SCVs) of pathogenic bacteria were discovered and described for the first time, way back in 1910 by Jacobsen in *Salmonella* Typhi.¹ They have subsequently also been demonstrated in *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, and the most constant defining feature of these variants is their colony size, which is about one-tenth of that of parent strains or "wild" strains.¹ They are getting renewed interest lately, due to their occurrence in chronic, debilitating infections.² *Staphylococcus aureus* SCVs are usually most frequently encountered among all SCVs, and are characterised by a reduced alpha-toxin expression, and are auxotrophic for various compounds like Menadione and thymidine.³ These morphological variants are very relevant clinically because they are highly resistant to most antibiotics, persist within host cells and evade the host immune response.⁴ Hence it is of utmost importance to know their prevalence in different samples and antibiogram, in order to administer correct antibiotic agents if need for empiric therapy arises. Keeping these things in mind, our study was aimed to see the prevalence, antibiogram and virulence factors of different bacteria showing small colony morphotype in a tertiary care hospital.

MATERIALS AND METHODS

This was a laboratory based observational study, carried out in the Department of Microbiology of the institute for a period of 1 year from September 2016 to August 2017. Different samples received

in the lab, were inoculated on routine culture media like Chocolate agar and CLED agar, and colonies were identified by conventional Gram staining and phenotypic and biochemical tests. Small colony morphotype was defined as any bacterium that had colony size 1/10th or less than the usual size of the wild type of that bacterium. Following identification, the following virulence factors were looked for in these bacteria: lecithinase, lipase and protease, based on colony appearance on Egg yolk agar (Nutrient agar 90 ml + 10 ml Egg yolk suspension). Lecithinase was denoted by presence of halo or opalescence around bacterial colonies, while protease was noted by a zone of clearing around the colonies. Lipase was defined by presence of pearly sheen on surface of colonies.

Antibiogram was carried out by Kirby-Bauer disk diffusion technique following CLSI protocol.⁵ For *S. aureus*, Amikacin (30 µg), Levofloxacin (5 µg), Clindamycin (2 µg), Cefoxitin (30 µg) and Azithromycin (15 µg) disks were used, while for Gram negative bacteria, Levofloxacin (5 µg), Nitrofurantoin 300µg (only for urinary isolates), Cefixime (30 µg), Ampicillin (10 µg) and Azithromycin (15 µg) disks were used (Himedia labs, India).

However we focussed mainly on the prevalence and virulence determinants. In case of SCV *Staphylococcus aureus*, identification was made by both slide and tube coagulase tests. Auxotrophicity in SCV *S. aureus* was determined by supplementing the bacterial colonies with Vitamin K, Carbon dioxide in candle jar, and subculturing on chocolate agar, in case

of various auxotrophic strains. Whenever an increase in colony size back to normal wild type was found after adding the

compounds, auxotrophicity for that compound was confirmed. All tests were carried out thrice with each isolate.

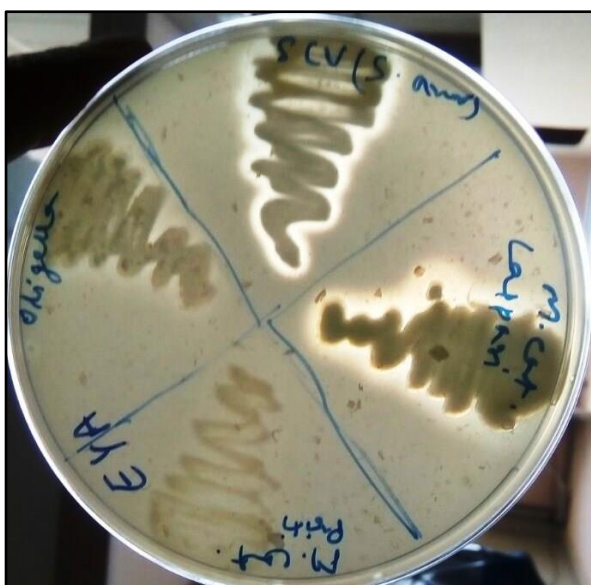
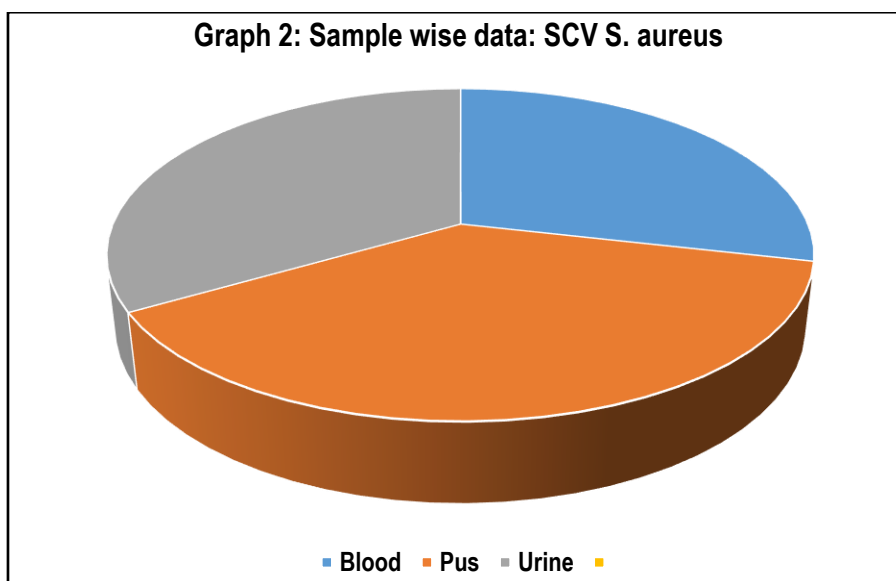
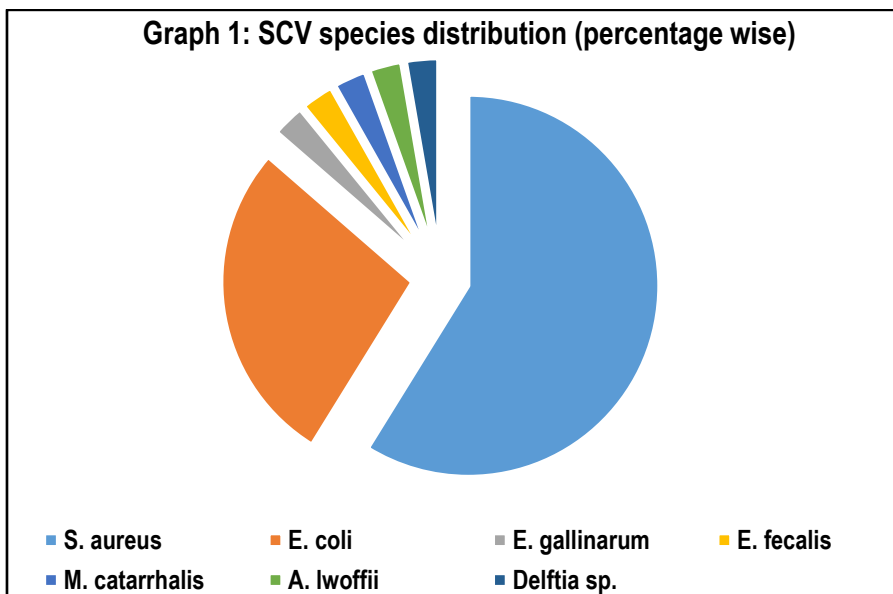


Fig 1: SCV *S. aureus* showing protease

RESULTS

In this period, about 4100 positive bacterial isolates grew in various samples, out of which only 36 were found to be SCV or small colony variants (0.8% of all samples). Mostly SCVs were found in *S. aureus* isolates, and 21 of those were retrieved (58.33% of all SCVs). Also the other bacteria showing SCV morphotype were *Escherichia coli* (10 isolates, 27.77% of all SCVs), and other bacteria like *Moraxella catarrhalis*, *Delftia tsuruhatensis*, *Acinetobacter Iwoffii*, *Enterococcus fecalis* and *Enterococcus gallinarum* (1 isolate each, 2.77% isolation for each). *S. aureus* SCVs were most commonly isolated from pus samples (8 cases) and also from urine (7 isolates) and blood samples (6 cases). However, *E. coli* SCVs were mostly recovered from urine (6 cases) and also from pus samples in 2 cases. *Pseudomonas aeruginosa* SCVs were not identified within this period. Interestingly, SCV *E. coli* was mostly isolated from female patients, while SCV *S. aureus* showed no such gender predilection. SCV *S. aureus* were mostly susceptible to Amikacin and usually refractory to Cefoxitin (33% strains of SCV *S. aureus*

were MRSA as tested by Cefoxitin disk method). SCV *E. coli* were usually resistant to Cephalosporins and Ampicillin, and susceptible to Amikacin. As regards virulence factors, *S. aureus* SCVs had lipase as the most common virulence factor followed by protease and lecithinase. In SCV of *E. coli*, the most common virulence factor was Lipase followed by Protease and lecithinase. Results have been shown in pie-charts 1 and 2. In SCV *S. aureus*, mostly they were auxotrophic to Hemin and Carbon dioxide.

DISCUSSION

Small colony variants arise from parent bacteria by genetic mutation and are very slowly growing with additional physiological characteristics.⁴ They have been described in different samples since long. After *S. Typhi*, *E. coli* SCVs were described way back in 1940s, and in these cases, subculture on glucose or tryptone containing media led to reversion to wild type *E. coli*.⁶ Mostly SCVs have been reported in *S. aureus*, and usually in our country as well as worldwide, they are reported in cases of chronic infections like Osteomyelitis and kidney disorders.⁷

Commonly SCVs arise from diminished Electron transport mechanism, as seen in *S. aureus*, *Enterococcus* spp. and *Pseudomonas aeruginosa*.⁸ Due to their small size of colonies, SCVs especially those of *S. aureus* are often missed or disregarded as commensal Diptheroids.⁹ As far as we know, there are very scanty reports of SCV *E. coli* from India so far. Only one report mentions isolation of SCV *E. coli* from South India, in a male patient, in a case of recurrent urinary tract infection.¹⁰ Also no study has addressed so far the virulence traits and antibiogram of these bacteria in our country which is also very important since in cases of high antibiotic resistance, anti-virulence therapies can be designed to kill or mitigate these pathogens which are notorious for persistence.

Another intriguing finding in our study is the uniform Amikacin susceptibility of SCV *S. aureus*, since they are usually reported to be resistant to aminoglycosides. This can be explained by the fact that our isolates were not Menadione or thiamine auxotrophs, and possibly hence did not lack all components of Electron transport chain. These aspects of SCVs are very interesting and should be followed by further studies.

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