

CASE REPORT

STAPHYLOCOCCUS HAEMOLYTICUS UNDER REPORTED BECAUSE OF FALSE POSITIVE SLIDE COAGULASE TEST: IMPLICATIONS FROM TWO CASE REPORTS

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ABSTRACT: Staphylococcus haemolyticus is a common coagulase negative staphylococcus (CONS) that is a commensal of the urethra and periurethral area in both sexes. It is feared for its multi-drug resistance and is associated with urinary tract infection (UTI), soft tissue infection and blood stream infection (BSI). Here we discuss two cases, one with urinary tract infection and another with urethral discharge presenting at the outdoor in our hospital. We isolated S. haemolyticus on culture in pure form from both cases, where after the identification was confirmed by Vitek2 compact AES. Slide coagulase test was misleading as both isolates were positive by this test rendering wrong reporting as S. aureus. Both isolates were confirmed to be CONS by tube coagulase test. Antibiotic susceptibility testing showed that multi drug resistance is not encountered in community acquired infection with S. haemolyticus. Slide coagulase test was misleading as both isolates were positive for the test rendering wrong reporting as S. aureus. Interpretation of susceptibility to different drugs, on the basis of this erroneous identification, leading to the suspicion of Methicillin and / or Vancomycin resistance, leads to the patients being advised ineffective drugs for clearing the infection. However, if tube coagulase test is taken into consideration and CONS is detected, as in our case, correct identification is made possible and correct treatment strategies may be devised.

KEYWORDS: Staphylococcus haemolyticus, Slide Coagulase, Community Acquired Infection.

INTRODUCTION: Staphylococcus haemolyticus is a coagulase negative staphylococcus (CONS) which is notorious for its multi-drug resistance.^{1,2,3} It is commonly present as commensal in the axilla, the perineum and the inguinal areas.⁴ It is a well-known opportunistic pathogen and is the second most frequently isolated CONS (S. epidermidis being the first).⁵ Infections are often associated with indwelling medical devices.^{6,7,8} Case reports of S. haemolyticus causing mainly nosocomial infections like urinary tract infection (UTI),⁹ blood stream infection (BSI),¹⁰ soft tissue infection,¹¹ have been documented. Many reports suggest S. haemolyticus to be the predominant CONS overriding S. epidermidis;^{12,13,14} and we support such reports and would like to emphasise that many S. haemolyticus isolates are masquerading as S. aureus because of false positive slide coagulase test. Aim of these case reports is to press upon the medical community the need for tube coagulase test as a routine and to correctly identify all CONS. In this era when patients are eager to study about their infection over internet it is also imperative to know the resistance level of community acquired S. haemolyticus infection.

CASE HISTORIES: First patient was a 32 yr old female attending surgery OPD for right sided loin pain since one week. There was neither any history of radiation of pain and haematuria. The second

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patient was a 34 yr male patient attending Gynaecology and STD OPD for treatment of infertility. On enquiring he admitted of occasional urethral discharge. Neither of the two had history of fever.

MATERIALS AND METHODS: Aseptically collected urine and urethral discharge respectively from the two patients were inoculated onto Mac Conkey agar and Blood agar. Colonies were then stained by Gram's stain. Catalase, Slide coagulase and tube coagulase test¹⁵ were done for identification of gram positive cocci. They were then tested by Vitek 2 compact Advanced Expert System (AES) for speciation. Antibiotic susceptibility testing was done by Kirby Bauer technique on Mueller Hinton Agar¹⁶ and interpreted according to CLSI 2014.¹⁷

RESULTS: After 24 hrs of aerobic incubation, Blood agar showed pure growth of colonies which were small (smaller than the typical yellow pin head colonies of *S. aureus*), white, moist, opaque, glistening, smooth with regular margin and with a narrow zone of β -haemolysis around the colonies. MacConkey's agar showed small lactose fermenting colonies. The colonies on Gram's stain showed gram positive cocci in clusters. In both cases the colonies were catalase and slide coagulase positive. Auto-agglutinability of the isolate was ruled out by using controls. In both cases tube coagulase test were negative at 2, 4 and 24 hrs. Vitek 2 compact AES speciated both colony isolates as *Staphylococcus haemolyticus* with 93 and 97% certainty respectively. On antibiotic susceptibility testing both were found to be resistant to Amoxycillin and Ciprofloxacin but sensitive to Amoxicillin-Clavulanate, Cefuroxime, Erythromycin, Clindamycin (D-Test negative), Gentamicin, Cotrimoxazole, Nitrofurantoin, Teichoplanin and Linezolid. Both were MSS (Methicillin susceptible *Staphylococcus*) by Cefoxitin disc test.

DISCUSSION: Most of the routine laboratories undertake slide coagulase test to identify catalase positive gram positive cocci in clusters. Speciation of CONS is performed by fewer laboratories. Ease of testing with much rapid result has made slide coagulase test a more common test adopted by most laboratories. It is a common practice in most laboratories to do tube coagulase test following only a negative slide coagulase test. Routine testing by both is seldom undertaken. In our experience this is leading to under-reporting of slide coagulase positive, tube coagulase negative CONS isolates and over-reporting of *S. aureus* isolates. Colony morphology should be given equal importance as that of coagulase reaction. Though tube coagulase test is time consuming, it should be undertaken whenever colony morphology raises suspicion of CONS. *S. lugdunensis*, *S. schleiferi* subsp. *schleiferi* are usually referred to as slide coagulase positive, but tube coagulase negative strains amongst CONS,¹⁵ and *S. Haemolyticus*, is always mentioned to be negative in both tests in all literatures. Ability of *S. haemolyticus* to give positive slide coagulase test changes many deductions. Interpretation of antibiotic susceptibility test result by disc diffusion changes dramatically. For e.g. a zone of 23 mm around cefoxitin is regarded as MSSA (Methicillin sensitive *Staphylococcus aureus*) for *S. aureus* whereas for the same zone it is MRS (Methicillin resistant *Staphylococcus*) for CONS.¹⁷ Vancomycin resistance cannot be commented upon in CONS. Wrong reporting as MSSA will result in prescribing β -lactams for treatment of these kinds of infections & hence leading to treatment failure. Moreover, ineffective treatment of *S. haemolyticus* infection will result in spread of antibiotic resistance amongst hospital strains because of its high propensity for gathering and spreading resistance genes.⁴ Prevalence of infections caused by *S. lugdunensis*, *S. schleiferi* subsp. *schleiferi* being quite

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less, misreporting does not cause statistically significant data modification. But, *S. haemolyticus*, being one of the major species of CONS that we come across clinically, under-reporting would cause decrease in prevalence rate of overall CONS as well as that of *S. haemolyticus* in causing infection. While different literatures mention high methicillin resistance and glycopeptide resistance in all *S. haemolyticus* isolates,^{18,19,20} antibiotic susceptibility test results for both of our cases show that antibiotic resistance is not high in community acquired infection by *S. haemolyticus*. Hence, identification as *S. haemolyticus* must not lead into over concern & over-zealous treatment with higher antibiotics. Treatment must be based on antibiotic susceptibility test result. Finally both our patients were treated with amoxicillin- clavulanate orally, thrice daily, for five days, with significant improvement in symptoms.

FUTURE PLAN: On the basis of our findings we now set up tube coagulase test regularly along with slide coagulase test for all gram positive cocci in clusters and any negative isolate in either test are speciated by Vitek 2 compact AES. Prevalence study of CONS in outdoor and indoor patient samples is ongoing.

REFERENCES:

1. Kunin, C. M., and C. Steele. Culture of the surfaces of urinary catheters to sample urethral flora and study the effect of antimicrobial therapy. *J. Clin. Microbiol.* 1985; 21: 902-908.
2. Gill, V. J., S. T. Selepak, and E. C. Williams. Species identification and antibiotic susceptibilities of coagulase-negative staphylococci isolated from clinical specimens. *J. Clin. Microbiol.* 1983; 18: 1314-1319.
3. Gruer, L. D., R. Bartlett, and G. A. J. Ayliffe. Species identification and antibiotic sensitivity of coagulase-negative staphylococci from CAPD peritonitis. *J. Antimicrob. Chemother.* 1984; 13: 577-583.
4. Fischetti, A.; Novick, R. P.; Ferretti, J. J.; Portnoy, D. A.; Rood, J. I.; Lina, G., Etienne, J., and Vandenesch, F. Gram-positive pathogens. Washington, D. C.: ASM Press; 2000. Chapter Biology and pathogenicity of staphylococci other than *Staphylococcus aureus* and *Staphylococcus epidermidis*; pp. 450-462. ISBN 978-1-55581-166-2.
5. deAlloriet al. Antimicrobial Resistance and Production of Biofilms in Clinical Isolates of Coagulase-Negative *Staphylococcus* Strains. *Biol. Pharm. Bull.* 2006; 29(8): 1592-1596. Doi:10.1248/bpb.29.1592.
6. Falcone et al. Teicoplanin use and emergence of *Staphylococcus haemolyticus*: is there a link? *Clin Microbiol Infect.* 2006; 12 (1): 96-97. Doi:10.1111/j.1469-0691.2005.01307.x. PMID 16460556.
7. Poyart et al. Rapid and Accurate Species-Level Identification of Coagulase-Negative Staphylococci by using the *sodA* Gene as a Target. *Journal of Clinical Microbiology (American society for Microbiology)*. 2001; 39 (12): 4296-4301. Doi:10.1128/JCM.39.12.4296-4301.2001. PMC 88539. PMID 11724835.
8. Viale, P.; Stefani, S. Vascular catheter-associated infections: a microbiological and therapeutic update. *J Chemother.* (Department of Medical and Morphological Research, Medical School, University of Udine, Italy) 2006; 18 (3): 235-49. Doi:10.1179/joc.2006.18.3.235. PMID 17129833.

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9. Bruce A. Gunn, Charles E. Davis, Jr. Staphylococcus haemolyticus Urinary Tract Infection in a Male Patient. *Journal of Clinical Microbiology*. May 1988; 26(5): p. 1055-1057.
10. L A Veach, M A Pfaller, M Barrett, F P Koontz and R P Wenzel. Vancomycin resistance in Staphylococcus haemolyticus causing colonization and bloodstream infection. *J. Clin. Microbiol.* September 1990; 28 (9): 2064-2068.
11. Adebayo Shittu, Johnson Lin, Donald Morrison and Deboye Kolawole. Isolation and molecular characterization of multi resistant Staphylococcus sciuri and Staphylococcus haemolyticus associated with skin and soft-tissue infections. *Journal of Medical Microbiology*. 2004; 53: 51-55.
12. Bares I, Herendi A, Lipcsey A, Bogнар C, Hashimoto H. Phage pattern and antibiotic resistance pattern of coagulase - negative staphylococci obtained from immunocompromized patients. *Microbiol Immunol*. 1992; 36: 947-59.
13. Guirguitzova B, Chankova D, Zozikov B. Staphylococci as uropathogens - frequency of isolation in hospitalized patients and sensitivity to antimicrobial agents. *Ann Urol (Paris)*. 2002; 36: 341-7.
14. Chaudhury A, Kumar A G. In vitro activity of antimicrobial agents against oxacillin resistant staphylococci with special reference to Staphylococcus haemolyticus. *Indian J Med Microbiol*. 2007; 25: 50-2.
15. Washington C. Winn Jr., Stephen D. Allen, W. M. Janda, Elmer W. Koneman, G. W. Procop, P. C. Schreckenberger and G. L. Woods, editors. *Koneman's color atlas and textbook of diagnostic microbiology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2008. Chapter 12, Gram - Positive Cocci, Part I: Staphylococci and Related Gram Positive Cocci; p. 623-671.
16. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition*. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
17. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing: twenty fourth Informational Supplement*. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
18. Santos Sanches I, Mato R, de Lencastre H, Tomasz A; CEM/NET Collaborators and the International Collaborators. Patterns of multi-drug resistance among methicillin resistant hospital isolates of coagulase - positive and coagulase- negative staphylococci collected in the international multi-centre study RESIST in 1997 and 1998. *Microb Drug Resist*. 2000; 6: 199-211.
19. Kumari N, Rai A, Jaiswal CP, Xess A, Shahi SK. Coagulase negative staphylococci as causative agents of urinary tract infections - prevalence and resistance status in IGIMS, Patna. *Indian J Pathol Microbiol*. 2001; 44: 415-9.
20. Paulsen IT, Firth N, Skurray RA. Resistance to antimicrobial agents other than β -lactams. In: Crossley KB, Archer GL, editors. *The Staphylococci in Human Diseases*, 1st ed. New York: Churchill Livingstone; 1997. p. 175-212.

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