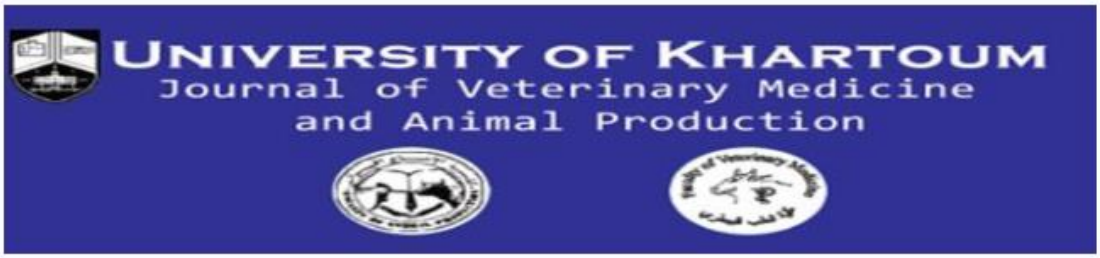
U. of K. J. Vet. Med. Anim. Prod., Vol.6, Issue 2 (2015) p 93-97

ISSN: 243456



A flow chart for the identification of *Staphylococcus* species

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Abstract

The genus *Staphylococcus* contains some species that are considered among the important pathogens of humans and animals. A chart as guide for the identification of species and subspecies of this genus was designed 20 years ago by El Sanousi and Said based on the information present in the ninth edition of Bergy's Manual for Determinative Bacteriology (1994). As new species have been identified since then, update of the chart was necessary. The present chart represents a comprehensive revision and update of the first one. The key tests of the first chart were kept, but some amendments and modifications were made to include entries for all species and subspecies that are listed under the genus *Staphylococcus* in the second edition of Bergy's manual of systematic bacteriology (2009). The present chart allows for identification of 53 staphylococci with a few numbers of tests.

المستخلص

يحتوى جنس المكورات العنقودية على بعض الأنواع التي تُعْتَبَرُ من بين المسببات المهمة للأمراض في الإنسان و الحيوان. و قد صمَّمَ السنوسي و سعيدٌ مخططاً مرشداً للتعَرُفِ على أنواع و نويعات هذا الجنس قبل عشرين عاماً، و ذلك بناءًا على ما احتواه دليل بيرجي لعلم البكتيريا التحديدي في طبعته التاسعة (1994) من معلومات. و منذ ذلك الحين فقد عُرِفتْ أنواعٌ جديدةٌ من هذه البكتيريا مما استدعى تحديثَ ذلك المخطط. يمثل المخطط الحالي مراجعةً شاملةً و تحديثاً للمخطط الأول. و إذْ أُبْقِيَ في المخطط الجديد على الاختبارات المفتاحية للمخطط الأول، فقد أُجْرِيَتْ عليه إصلاحاتٌ و تعديلاتٌ لإضافةِ مدخلاتٍ جديدةٍ لكل أنواع و نويعات جنس المكورات العنقودية التي ذُكِرَتْ في الطبعة الثانية لدليل بيرجي لعلم البكتيريا النظامي (2009). و بهذا، يتيح المخطط الحالي التعرف على 53 من هذه المكورات العنقودية باستخدام عددٍ قليلٍ من الاختبارات.

Introduction

The genus *Staphylococcus* contains some of the most important bacterial species that infect humans and animals, as well as species important in food microbiology. At present, there are 51 approved and proposed *Staphylococcus* species, ten of which have two, three or four subspecies (https://en.wikipedia.org/wiki/Staphylococcus. Identification of and differentiation between members of this genus using standard tables of biochemical and physiological tests is time consuming. Using commercially available identification kits, such API system, is advantageous, but it is very expensive and not affordable for many young researchers and postgraduate students in the developing countries, such as the Sudan. A scheme for the identification of *Staphylococcus* species was developed in 1996 by El Sanousi and Saeed (S. M. El Sanousi, personal communication) and was used as guide at the bacteriology laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, since then. The chart was designed from data on the staphylococci contained in the 9th edition of Bergy's Manual of Determinative Bacteriology (Holt *et al.*, 1994), which was based on the tables prepared by Kloos and Schleifer (1986) in the first edition of Bergy's Manual of Systematic Bacteriology (Holt *et al*., 1986) in addition to amendments with data published later. The chart was modified twice by inclusion of entries for a few species and subspecies that were described thereafter. As many new staphylococci have been described in the second edition of Bergy's Manual (Schleifer and Bell, 2009), an update of the chart was necessary. The present chart represents a comprehensive revision and update of the first one. The key tests of the first scheme were kept, but some amendments and modifications were made to include entries for all staphylococci that are listed in the second edition of Bergy's Manual (Schleifer and Bell, 2009).

Description of the chart

The chart represents a guide for the identification of the species of an organism identified to belong to the genus *Staphylococcus*. The chart includes few numbers of biochemical and physiological tests instead of using all tests listed in the tables of identification. These tests represent the most important and key tests that can be used to differentiate between the species and subspecies listed under the genus *Staphylo-coccus*, that were included in the second edition of Bergy's manual of systematic bacteriology (Schleifer and Bell, 2009). Three key tests are used in sequence in the chart to differentiate the species and subspecies of the genus *Staphylococcus* into 4 groups contained into 4 boxes. These key tests are: coagulase activity, resistance to novobiocin and the aerobic production of acid from mannose. The first key test divides staphylococci into two main groups: coagulase positive staphylococci (CPS) and coagulase negative staphylococci (CNS). The second key test divides the coagulase negative staphylococci into novobiocin sensitive (CNS-NS) and novobiocin resistant (CNS-NR) subgroups. As all novobiocin sensitive staphylococci are oxidase negative, oxidase test is used for this group to exclude *Macrococcus caseolyticus* (formerly, *Staphylococcus caseolyticus*). Then, the third key test is used to divide the coagulase negative – novobiocin sensitive

ISSN: 243456

staphylococci into two further subgroups: mannose positive (CNS-NS-MP) and mannose negative (CNS-NS-MN) subgroups. According to these key tests, the flow chart leads to the 4 boxes: the first box contains the coagulase positive staphylococci (CPS), the second box contains the coagulase negative – novobiocin resistant staphylococci (CNS-NR), the third box contains the coagulase negative – novobiocin sensitive – mannose negative staphylococci (CNS-NS-MN), and the fourth box contains the coagulase negative – novobiocin sensitive – mannose positive staphylococci (CNS-NS-MP). Within each box, differential characters used are mainly the aerobic production of acid from some carbohydrates in addition to few other characters including aerobic or anaerobic growth, pigment production, colony size and activity for oxidase, urease, glucourindase and nitrate. In Table 74 (Characteristics differentiating the species and subspecies of the genus *Staphylococcus*) in volume 2 of the second edition of Bergy's Manual of Systematic Bacteriology (De Vos *et al*., 2009), the symbols "+" or "–" were used to indicate that 90% or more of the strains are positive or negative, respectively; the "d" indicates that 11 – 89% of the strains are positive, the brackets ( ) indicates a delayed reaction and "w" indicates a weak reaction. In this chart the same symbols are used except the symbol "d", where "+" stands for either "+" or "d". When a species has the symbol "d" for one of the key tests, two entries are made for it in the chart. *S. hyicus* has a "d" symbol in the coagulase test and, accordingly, it has two entries in the chart: one in the direction of coagulase positive box (CPS) and the other in the direction of the coagulase negative (CNS) boxes. The same was applied for *S. simulans* regarding the production of acid from mannose, and so it has two entries to the boxes (CNS-NR-MP) and (CNS-NR-MN). The information on *S. condimenti* regarding the resistance to novobiocin, oxidase and xylose was not determined (ND) in this edition of Bergy's Manual. Available information regarding novobiocin resistance, as well as xylose, for this species provided by Resch *et al*. (2008) and Misawa *et al*. (2014) were used in the chart. Accordingly, *S. condimenti* has two entries: one in the direction of novobiocin sensitive (CNS-NR-MP) box and the other in the direction of novobiocin resistant (CNS-NR-MP) box. In the later box, entry for *S. condimenti* was made in the direction of oxidase negative. It is noteworthy that *S. simiae* and *S. pseudointermedius* were not listed in Table 74, but they were mentioned at the end of the description of the genus *Staphylococcus* (Schleifer and Bell, 2009) as addendum. The carbohydrates included for testing in the chart are: fructose, lactose, maltose, mannitol, mannose, melezitose, raffinose, ribose, salicin, sucrose, trehalsoe.

How to use the chart?

It is assumed that persons who use this chart will have sufficient information and experience on general bacteriology techniques and, accordingly, are able to perform the physiological and biochemical tests used in the identification of bacteria properly. So, after performing proper tests indicating that the isolate under test belongs to the genus *Staphylococcus*, the first step to do is to test the isolate for coagulase activity. If the isolate is positive for coagulase, the flow chart leads to

the box CPS. Inside this box, the first step is to test the organism for the production of acid from xylose. A positive result means the isolate is *Staphylococcus lutrae*. A negative result for xylose leads to test the organism for pigment production and fermentation of mannitol (anaerobically). Positive result indicates the isolate is *S. aureus* subsp. *aureus*. Negative results leads to incubate the isolate aerobically. No growth means the isolate is *S. aureus* subsp. *anaerobius*. When positive aerobic growth result is obtained, test results of the isolate for the production of acid from maltose and trehalos will assign the isolate to one of these species: *S. intermedius*, *S. pseudointermedius*, *S. delphini*, *S. hyicus* and *S. schleiferi* subsp. *coagulans*.

When coagulase activity test result is negative, the isolate should be tested for novobiocin resistance. If it is novobiocin sensitive, the isolate should be tested for oxidase activity to exclude *Macrococcus caseolyticus*, which was formerly known as *S. caseolyticus*. Then, the oxidase negative isolate should be tested for the production of acid from mannose. According to the result, the flow chart leads into either the box CNS-NS MP or the box CNS-NS-MN. Inside each box a number of tests will be applied in sequence to assign the isolate to one of the species or subspecies. If the isolate is novobiocin resistant the flow chart leads CNS-NR box, inside which the first test is for the oxidase activity followed by a few number of tests, mostly are for carbohydrate utilization.

In conclusion, the chart allows for identification of 53 staphylococci with a few numbers of tests.

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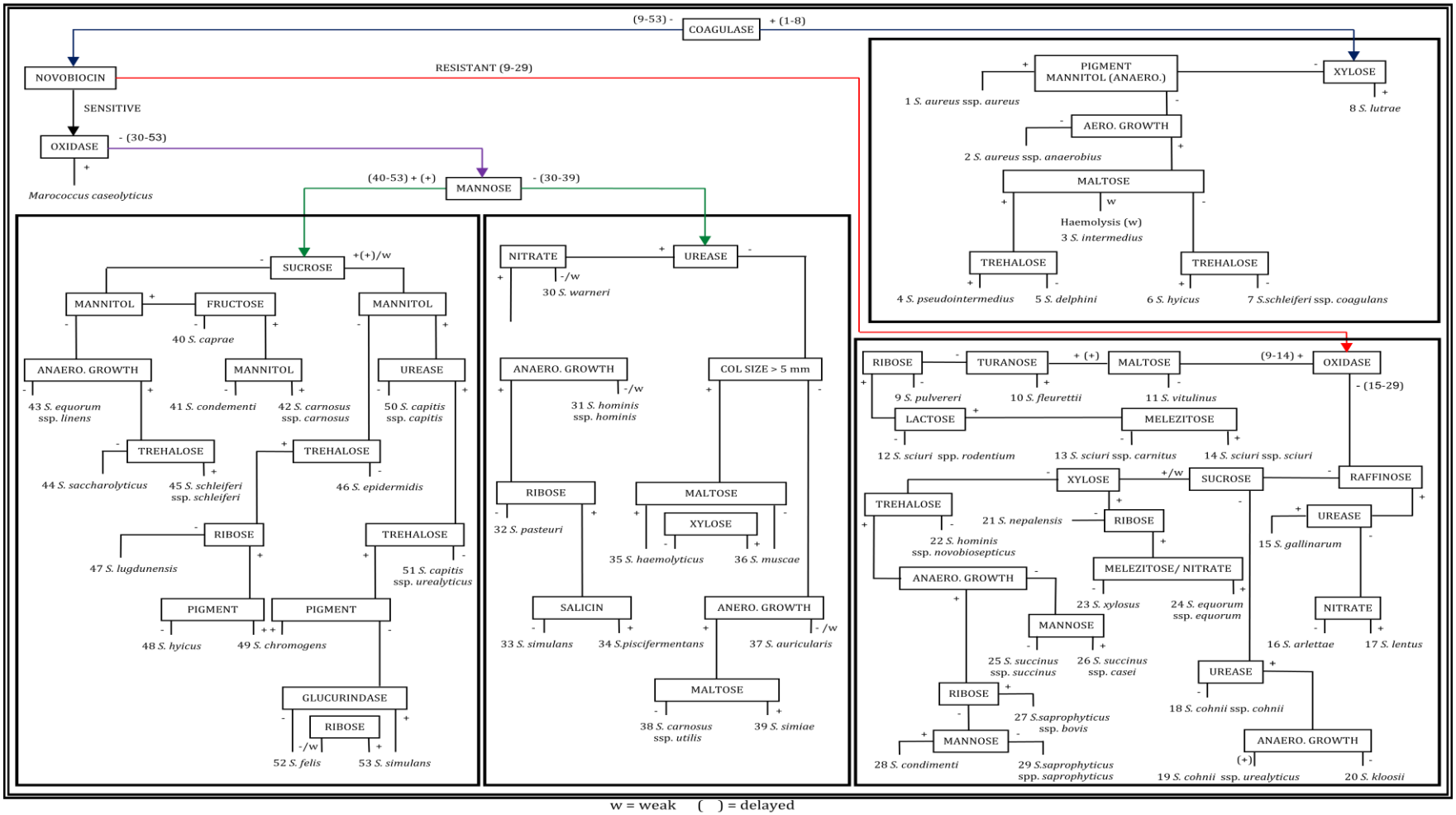

w = weak ( ) = delayed