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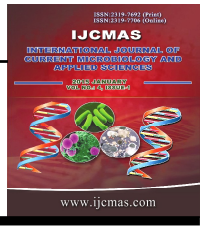

Original Research Article

Isolation and characterization of *Staphylococcus aureus* in spoiled food samples

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Keywords

Staphylococcus aureus, strains, Plasmid

A B S T R A C T

To investigate the incidence and antibiotic resistance of *Staphylococcus aureus* strains isolated from skin, nose and throat of individuals and to trace the ecological origin of the *Staphylococcus aureus* isolated. Three smears aseptically collected on a random basis from skin, nose and throat of healthy individuals were analyzed for the presence of *Staphylococcus aureus*. Isolates were identified by Phenotyping methods and Plasmid analysis. The identification results showed that a predominance of *Staphylococcus aureus* in all samples and from three different isolates there are two *Staphylococcal* strains were analyzed for their plasmid similarities and plasmid associated resistance determinants.

Introduction

A phenotype (*from Greek phainein, 'to show' + typos, 'type'*) is the composite of an organism's observable characteristics or traits, such as its morphology, development, biochemical or physio-logical properties, phenology, behavior, and products of behavior (such as a bird's nest) (Crusio WE 2002). A phenotype results from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two. When two or more clearly different phenotypes exist in the same population of a species, the species is called polymorph (Tabery 2007).

The genotype of an organism is the inherited instructions it carries within its genetic code. Not all organisms with the same genotype look or act the same way because appearance and behavior are modified by environmental and developmental conditions. Likewise, not all organisms that look alike necessarily have the same genotype (O'Brien et. al 2002). This genotype-phenotype distinction was proposed by Wilhelm Johannsen in 1911 to make clear the difference between an organism's heredity and what that heredity produces (Tabery et. al 2010). The distinction is similar to that proposed by August Weismann, who distinguished between germ plasm (heredity) and somatic cells (the body) (O'Brien et. al 2002). The genotype-phenotype distinc-tion should not be confused with Francis Crick's central

dogma of molecular biology, which is a statement about the directionality of molecular sequential information flowing from DNA to protein, and not the reverse (Crusio WE 2002).

Gene–environment interaction (or genotype–environment interaction or G×E) is the phenotypic effect of interactions between genes and the environment (Thomas et.al 2008). It is exploited by plant and animal breeders to benefit agriculture. For example, plants can be bred to have tolerance for specific environments, such as high or low water availability. The way that trait expression varies across a range of environments for a given genotype is called its norm of reaction (Tabery 2007).

The knowledge that DNA is the genetic material for cells came from studies on transformation by Griffith and Avery and from experiments on T2 phage reproduction by Hershey and Chase but the complete DNA sequence of an organism does not contain the information necessary to specify the organism (Crusio WE 2002). The outcome of developmental processes depends both on the genotype and on the temporal sequence of environments in which the organism develops (Tabery et. al 2010). Many experiments on many different organisms where it has been possible to produce multiple individuals of the same genotype show this same result (Tabery 2007).

Bacteria for example may also contain a small DNA molecule called plasmid that is physically separate from, and can replicate independently of, chromosomal DNA within a cell. Most commonly found as small circular, double-stranded DNA molecules in bacteria, plasmids are sometimes present in archaea and eukaryotic organisms (Tabery et. al 2010). In nature, plasmids carry genes that may benefit survival of the organism (e.g. antibiotic resistance), and can frequently be transmitted from one bacterium to another (even of another species) via horizontal gene transfer (Thomas et.al 2008).

By function, the plasmids can classify into five main classes; Fertility (F) plasmids, which contain *tra* genes. They are capable of conjugation and result in the expression of sex pilli, Resistance (R) plasmids, which contain genes that provide resistance against antibiotics or poisons (Tabery 2007). Historically known as R-factors, before the nature of plasmids was understood, Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria, Degradative plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid and Virulence plasmids, which turn the bacterium into a pathogen (Lipps 2008). Plasmids can belong to more than one of these functional groups (Thomas et.al 2008).

Materials and Methods

Samples

Numerous species of *Staphylococ-cus*, including *Staphylococcus aureus* isolated from the skin, nose and throat of healthy individuals. This accounts for its presence in foods, primarily those which not heated after preparation which involved handling, such as cream puffs or potato salad. There is also a high carrier rate for *Staphylococcus aureus* in hospital personnel, which explains the high incidence of the organism in "nosocomial" infections. Nosocomial infections are infections that develop in the course of a hospital stay and were not present in the patients upon admission to the hospital.

Isolation and identification methods of the bacteria:

The isolates were cultured onto nutrient agar and incubated for 24-48 hours at 37°C (Acco M. et. al 2003). Suspected isolates were identified primarily as white, circular convex, entire colonies. Pure culturing was done on Nutrient agar by quadrant streaking and then sub- cultured on Trypticase soy agar and blood agar for Colony morphology and other examinations (Oyeleke et. al 2008).

Biochemical examination:

Biochemical tests were performed to confirm *Staphylococcus aureus* using Catalase test, Coagulase test and Carbohydrate fermentation test (Acco M. et. al 2003).

Determination of susceptibility of *Staphylococcus aureus* isolates to 6 antibacterial agents:

The susceptibility of isolates to different anti-microbial agents [Strepto-mycin (30 µg), Tetracycline (30µg), Nalidixic acid (30 µg), Gentamycin (30 µg), Chloramphenicol (30 µg), Ampicillin (10 µg)] was done by well diffusion testing (WD), a diffusion method similar to disc diffusion. Bacteria are suspended in saline and streaked on Mueller Hinton agar. Plates were incubated for 24 hours at 37^{o}C (Acco M. et. al 2003). Characteriza-tion of strains as sensitive, intermediate or resistant was based on the size of zones of inhibition surrounding the wells (Oyeleke et. al 2008).

Plasmid extraction

Using three to five well isolated Colonies; inoculate one tube of Growth medium for each test Strain and Control Strain to be screened. For *Staphylococcus aureus*, use 20 ml of Muller-Hinton Broth divided in to two 10 ml cultures. Small nucleic acids are better separated by polyacrylamide gels; large DNA molecules are only able to move end-on in a process called "reptation" and are more difficult to separate (Soomro AH et. al 2003). In general lower concentrations of agarose are better for larger molecules; it will exaggerate the distances between bands (Normanno G. et. al 2005).

The disadvantage of higher concentrations is the long run times (sometimes days). Instead these gels should be run with a pulsed field electrophoresis (PFE), or field inversion electrophoresis. A DNA or RNA band visible for agarose gel electro-phoresis is ethidium bromide, usually abbreviated as EtBr (Acco M. et. al 2003). It fluoresces under UV light when intercalated into DNA (or RNA) (Normanno G. et. al 2005).

Electrophoresis Buffer

Several different buffers have been recommended for electrophoresis of DNA. The most commonly used for duplex DNA are TAE (Tris-acetate-EDTA) and TBE (Tris-borate- EDTA) (Normanno G. et. al 2005). DNA fragments will migrate at somewhat different rates in these two buffers due to differences in ionic strength. Buffers not only establish a pH, but provide ions to support conductivity (Le Loir Y. et. al 2003).

Ethidium bromide

A fluorescent dye used for staining nucleic acids. Note: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical-wear glove while handling (Soomro AH et. al 2003).

Transilluminator
(an ultraviolet light box):

Is used to visualize ethidium bromide-stained DNA in gels. Note: Always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light (Soomro AH et. al 2003).

After electrophoresis the gel is illuminated with an ultraviolet lamp (usually by placing it on a light box, while using protective gear to limit exposure to ultraviolet radiation) to view the plasmid DNA bands (Oyeleke et. al 2008). The ethidium bromide fluoresces reddish- orange in the presence of plasmid DNA. The plasmid DNA band can also be cut out of the gel, and can then be dissolved to retrieve the purified plasmid DNA (Acco M. et. al 2003). The gel can then be photographed usually with a digital or polaroid camera. Although the stained nucleic acid fluoresces reddish-orange, images are usually shown in black and white (Le Loir Y. et. al 2003).

Results and Discussion

The Phenotyping methods for characterization and identification Incl-ude: Isolation of *Staphylococcus aureus*, Pure culturing, Colony morphology (Figure 1, 2 and 3), Gram staining, Motility, Spores (Endospores) staining, Catalase test, Coagulase test, Carbo-hydrate fermentation test (glucose, mannitol), Antibiotic sensitivity which give the basic or general characteristics of the *Staphylococcus aureus* are described in Table 1.

The genotyping methods including plasmid DNA samples analysis of three different isolates are described in figure 4.

1, 2, and 3: Isolates collected from different individuals. Based on Restriction endonuclease analysis of plasmid DNA, these strains were grouped as:

Isolates 1and 2 are identical to each other.

Isolate 3 is not related to isolates 1and 2.

Most bacterial species harbor plasmids, although this is not a universal feature of prokaryotes. For the purpose of strain typing, however the function of the plasmid or the genes it carries is irrelevant.

Figure.1 Staphylococcus aureus on nutrient agar

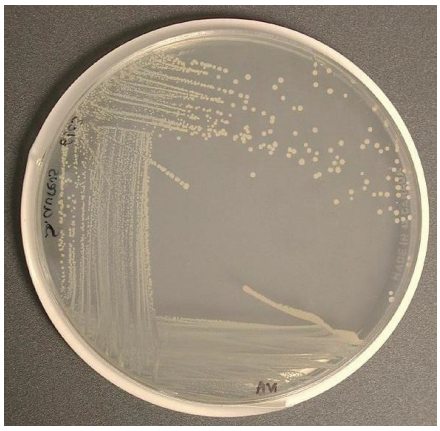


Table.1 Phenotyping methods for Isolation and identification of the Staphylococcus aureus from smears aseptically collected on a random basis from skin, nose and throat of healthy individuals.

No.	Test	Result
1.	Isolation of Staphylococcus aureus	On nutrient agar white, circular convex, entire colonies were found.
2.	Pure culturing	Single isolated colonies were found on Nutrient agar by quadrant streaking.
3.	Colony morphology	On Trypticase soy agar, circular, pinhead colonies are seen, on nutrient agar white, circular, entire, convex colonies are seen, and on blood agar a hemolysis of the agar in the area around the colony is seen.
4.	Gram staining	Gram positive cocci were observed under oil immersion lens.
5.	Motility	By hanging drop method found that the strain is motile.
6.	Endospore staining	Non endospore formers were observed.
7.	Catalase test	Effervesces were observed in all the isolates.
8.	Coagulase test	Visible clumping was observed in all the cultures.
9.	Carbohydrate fermentation	All the isolates are acid positive.
10.	Antibiotic sensitivity test	A zone of inhibition was found against ampicillin and tetracycline.

Figure.2 *Staphylococcus aureus* on Trypticase soy agar

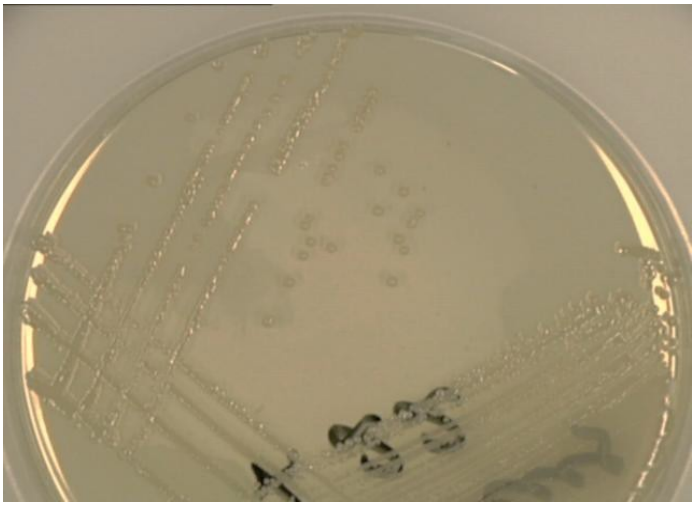


Figure.3 *Staphylococcus aureus* on blood agar

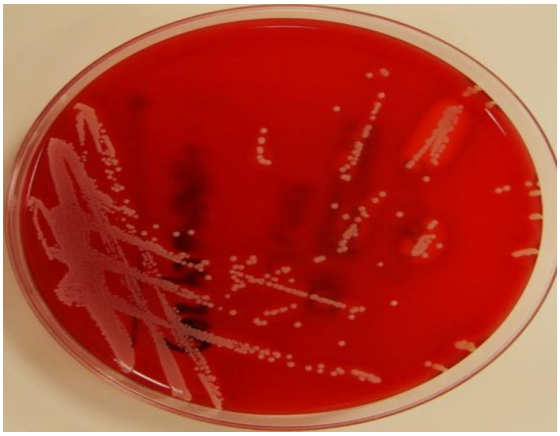
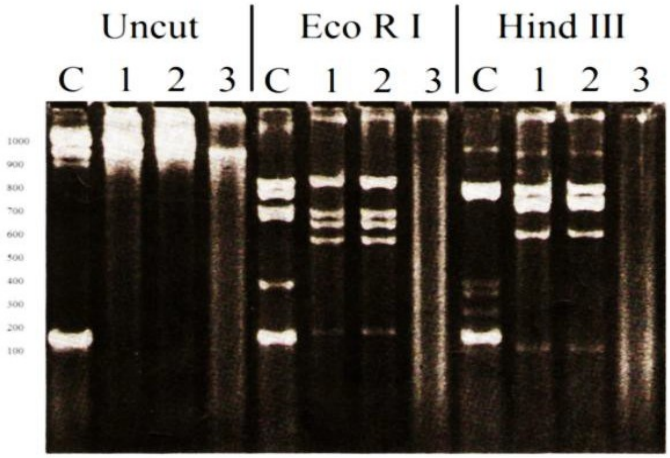


Figure.4 Agarose gel electrophoresis of digested plasmid DNA samples (lane 1 from skin, lane 2 from nose, lane 3 from throat). Lane C: control strain. Uncut: undigested plasmid DNA, EcoR1: DNA digested with Eco R1, Hind111: DNA digested with Hind111



Rather it is the numbers and sizes of the plasmids present in the isolates are critical. The number of different plasmids of different sizes carried by an organism varies from species to species. The greater the number of plasmids the easier is to identify that particular strain among a collection of isolates in an outbreak investigation. Plasmid analysis is based on the fact that different bacterial strains often carry different types or number of plasmids. Plasmid DNA is isolated from the bacterial cells, digested with restriction endonuclease and electrophoresed through agarose.

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