

Emergence of MDR *Aerococcus Viridans* in Diarrhea in Chicken in Egypt as First Study

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Abstract

Aerococcus spp. thought to be a harmless pathogen, it is now recognized as a promising disease-causing microorganism. In the field of microbiology, *Aerococci* is frequently confused with streptococci, leading to incorrect diagnoses. Antimicrobial resistance is globally increasing which is reflected in public health threats. Therefore, the current study was performed to evaluate the incidence of *A. viridans* in one hundred fecal swabs collected from broilers and layers farms in the Sharkia government, Egypt, and described its antibiotic resistance by the VITEK system. Only one *A. viridans* isolate was identified from 100 samples. *A. viridans* isolate was susceptible to vancomycin, Trimethoprim/ sulfamethoxazole, and linezolid, while, *A. viridans* isolate was resistant to Penicillin G, meropenem, tetracycline, and Ceftriaxone. As the first study in Egypt, this study concluded that MDR *A. viridans* is of importance in public health and plays an essential role in diarrhea infection in poultry, and is considered a hazard to human health.

Key words: *Aerococcus viridans*, MDR, VITEK, Poultry, Diarrhea

Introduction

Diarrhea is one of the most serious illnesses that affect poultry production and causes high mortality rates, a decline in egg production, a reduction in feed conversion, carcass condemnations, and other losses in the world's poultry industry, resulting in significant annual losses (Dziva and

Steven, 2008 and Barnes et al., 2008).

Aerococci is often confused with *streptococci* because they have the same biochemical and physiological characteristics as *pediococci*, *enterococci*, *lactococci*, and *leuconostocs* (Facklam et al., 1989). This genus responds weakly to the catalase test, but it doesn't

have any cytochrome. *Aerococcus viridans* is the only species of the genus *Aerococcus* that has been described and named (*Williams et al., 1953*).

According to (*Spakova et al., 2012*), these organisms are currently considered to be of significant importance in both human and veterinary medicine. *A. viridans* is responsible for many human health risks, including endocarditis, meningitis, and arthritis according to (*Gopalachar et al., 2004; Popescu et al., 2005*). *A. viridans* was recently described as the causative agent of arthritis, pneumonia, and meningitis in cows. *A. viridans* was recently involved in bovine mastitis (*Liu et al., 2015*). *A. viridans* maintains to be one of the most significant hazards to the rural economies of numerous countries, including Egypt, and it is one of the infectious diseases that affect large ruminants. The occurrences in Egypt, there is just a limited amount of scientific research available (*Shaker et al., 2019*). The current study aims to screen the role of MDR *A. viridans* in cases of diarrhea in poultry in Egypt, as the first study in Egypt, and to describe the bacteria's resistance to antimicrobials.

Materials and methods

Sample Collection

The study was carried out between January 2022 to March 2022 on one hundred chicken fecal samples that were randomly collected from

different poultry farms (broilers & layers) in the Sharkia government. All samples were collected under complete aseptic conditions in sterile plastic swabs and transported to Animal Health Research Institute, Ismailia lab. on an icebox for microbiological examination.

Isolation of *Aerococcus viridans*

All samples were examined to determine the presence of *Aerococcus viridans*, which were isolated and identified. (*Sun et al., 2017*). The samples were inoculated on trypticase soya agar (Sigma, Shanghai, China) with 5% sheep blood, nutrient agar, and CLED agar, and the plates were incubated at 37 °C for 24 hours.

Aerococcus viridans Identification

Microscopical examination of films made from pure cultures and stained with Gram-stain, All cultures that did not produce a positive catalase reaction were classified as prospective *Aerococcus* isolates and were kept for further identification using the VITEK2 system (bioMérieux, SA, France).

Antibiogram Testing

Antimicrobial sensitivity of *A. viridans* isolates according to (*CLSI, 2013*) guidelines against seven antimicrobial agents categorized into seven classes was performed utilizing the VITEK2 (bioMérieux, SA, France) according to the manufacturer's instructions.

Results

Prevalence of *A. viridans*

Only one isolate(1/100,1%) was obtained in this study. On nutrient agar appeared as small, semi-transparent, white or gray colonies, and on Sheep blood agar appeared as circular, alpha-hemolytic colonies Figure(1) while on CLED media appeared as small yellowish colonies Figure (2). Microscopically, The organisms resemble Gram-positive cocci that are 1-2 nm in diameter and stained deeply and can be dispersed singly, in pairs, in tetrads, or other irregular clusters, non-sporulated and non-

capsulated. Biochemically, *A. viridans* is characterized by negative in catalase and detected by VITEK2 as shown in Table (1)

Antibiogram Testing

The result of the antibiogram of one strain isolated from chicken. **Table (2)** revealed that one isolate was resistant to tetracycline, penicillin G , meropenem, and ceftriaxone and sensitive to vancomycin, linezolid, and Trimethoprim/ sulfamethoxazole and considered as MDR. MARI was determined as 0.43

Table (1): The results of different biochemical tests on *Aerococcus viridans*

No.	Test	<i>Aerococcus viridans</i>
1	Gram staining	Gram-positive
2	Catalase	-
3	Oxidase	-
4	Coagulase	-
5	Indole	-
6	Urease	-
7	Citrate Utilization	+
8	Nitrate reduction	-
9	Methyl red	+
10	Vogues proskure	-
11	Maltose fermentation	+
12	Glucose fermentation	+
13	Mannitol fermentation	variable

Table (2): The results of the antibiogram sensitivity test for the examined *Aerococcus viridans*

Antimicrobial agent	Type of sensitivity	MIC
Penicillin	R	=2
Meropenem	R	>1
Ceftriaxone	R	>4
Linezolid	S	=2
Vancomycin	S	=1
Tetracycline	R	>8
Trimethoprim/ sulfamethoxazole	S	<0.5/9.5

S= susceptible R=resistant



Figure (1): *Aerococcus viridans* on Sheep Blood Agar



Figure (2): *Aerococcus viridans* on CLED media

Discussion

Earlier investigations revealed a low incidence of *A. viridans* in bovine mastitis, pneumonia, and diarrhea, which may have been due to misidentifications as streptococci and staphylococci (*Rasmussen, 2013*). The detection of *A. viridans*, on the other hand, has become more reliable as a result of developments in identification methods (*Sun et al., 2017*). According to the findings of (*Liu et al., 2015*), the rate of

isolation of *A. viridans* from cases of subclinical mastitis in Northern China was 6.1%. (*Saishu et al., 2015*) reported the incidence of *A. viridans* in clinical mastitis cases in Japan was 8% nearly similar to our results. Eleven strains have been isolated from subclinical mastitis milk samples from dairy farms in Egypt with 11% (*Shaker et al., 2019*) higher than our results. There are several automated technologies for identifying and

assessing susceptibility to the most frequent bacteria in medical settings (*Joyanes et al., 2000*). Originally, the VITEK system (bioMérieux-Vitek, Hazelwood, Missouri) was intended as an onboard device for the detection and identification of urinary tract diseases from astronauts in spacecraft. 1979 marked its introduction in clinical laboratories, and since then it has been extensively evaluated (*Doern et al., 1979*).

A. viridans isolate was resistant to tetracycline, penicillin G, meropenem, and ceftriaxone and sensitive to vancomycin, linezolid, and Trimethoprim/sulfamethoxazole and considered as MDR. MARI was determined as 0.43. The same findings were reported by (*Paková et al., 2012*), particularly regarding the resistance patterns of beta-lactamase resistance. On the other hand, (*Martin et al., 2007*) demonstrated a different finding, as they discovered that all *A. viridans* isolates were able to be treated with B-lactamase antibiotics. The fact that *A. viridans* is resistant to some commercial antibiotics, including those that are frequently used in various treatments programmed for animals and humans, lowers the efficacy of antibiotics against infections and is attributed to be a threat to humans.

In conclusion, our results *A. viridans* play a role as one of the causative agents of diarrhea in poultry as a first report in Egypt and are

respected as a hazard for human infections with *A. viridans*.

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الملخص العربي

يُعتقد أن *Aerococcus* spp. غير ضار ، فقد تم التعرف عليه الآن على أنه كائن حي دقيق واعد يسبب المرض. لذلك تم إجراء الدراسة الحالية لفحص معدل الإصابة بـ *A. viridans* في 100 مسحة برازية تم جمعها من مزارع الفرايج والبياض في محافظة الشرقية بمصر ، وقد تم وصف مقاومتها للمضادات الحيوية. تم تحديد عينة واحدة فقط من عزلة *A. viridans* من 100 عينة. كانت عزلة *A. viridans* حساسة لفانكوميسين ، Trimethoprim / sulfamethoxazole و لينزوليد ، بينما عزلة *A. viridans* كانت مقاومة للبنسلين G ، ميروبنيم ، تيتراسيكلين و سيفترياكسون. خلصت هذه الدراسة كأول دراسة في مصر إلى أن *A. viridans* تلعب دوراً أساسياً في الإصابة بالإسهال في الدواجن وتعتبر خطراً على صحة الإنسان.