
Automated ID & AST using VITEK 2 and Prevalence of *K. pneumoniae* Isolated from diarrheic chicken in Sharqiyah Governorate

Ahmed R. Khafagy¹, Nada H. Eedarous¹, Waleed F. Khalil², Fatma M. Youssef³, Mona A. Ahmed⁴ and Fatma El-Zahraa S. Mohammed⁵

1 Department of Bacteriology, Immunology, and Mycology Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt

2 Department of Pharmacology Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt

3 Chief researcher of pathology department at animal health research institute, Ismailia.

4 Chief researcher of bacteriology department at animal health research institute, Ismailia.

5 Free Veterinarian

Abstract

The present study was conducted to identify and isolate the bacterial pathogens that cause gastrointestinal problems in chickens, leading to a decrease in their production. The research also aimed to determine the incidence of these pathogens and their sensitivity to antibiotics. To achieve this, one hundred samples were collected from the cloaca of chickens suffering from diarrhea in four different private farms located in Bilbies Sharqiyah Governorate and were analyzed using bacteriological methods. The results revealed that there were 5 isolates: 39% *E. coli*, 22% *C. perfringens*, 14% *K. pneumoniae* and 14% *P. mirabilis*, 11% *P. vulgaris* along with no detection of Salmonellae (0%). All 14 *K. pneumoniae* isolates previously identified using the traditional methods were identified rapidly and automatically by VITEK 2 system (bioMerieux, Craponne, France) using AST and ID-GNB cards, Vitek 2 software, Advanced Expert System [AES] software, and all 14 isolates were sensitive for 24 different antimicrobial agents: Cefazolin, Cefepime, Ciprofloxacin, Gentamycin, Tobramycin, Cefuroxime, Piperacillin, Amikacin, Meropenem, Cefotaxime, Trimethoprim sulfa, Ceftazidime, Levofloxacin, Chloramphenicol, Imipenem, Aztreonam, Cefoxitin, Amoxicillin/CA, Ticarcillin/CA, Minocycline, Ertapenem, Tigecycline, Moxifloxacin, Ampicillin/sulbactam.

Key words: *E. coli*, *C. perfringens*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris* & VITEK 2 automated system

Introduction

The poultry sector is significant in contributing to the country's income as it involves raising domesticated birds for their meat, eggs, and edible parts. Poultry, particularly chicken, is one of the most consumed meats globally. However, poultry is susceptible to various diseases caused by bacteria, which can cause high rates of death and illness among the birds, mostly affecting their digestive and respiratory systems (*Yegani and Korver, 2008*).

Enterobacteriaceae, which is a type of bacteria that has a negative gram stain, can be found in various places such as plants, water, soil, and the digestive tracts of animals. They can cause disease by attacking their host using several methods, including colonization factors, motility, endotoxin, and enterotoxin. These factors are crucial for the bacteria to successfully infect their host (*Markey et al., (2013)*).

Klebsiella bacteria are a type of Gram-negative bacteria that have a protective outer layer and a rod-like shape. These bacteria are considered opportunistic because they can cause severe illnesses in humans and animals, particularly if they have weakened immune systems. Klebsiella bacteria can be spread from person to person. The most common pathogenic species within the Klebsiella genus is *K. pneumoniae*. Klebsiella bacteria are part of the Enterobacteriaceae family and are characterized by their size, which can range from 1.0 to 1.0 mm

in width and 0.6 to 6.0 mm in length. They often form colonies with a slimy or mucoid appearance. Klebsiella bacteria have 77 different types of antigens on their surface, which can result in a variety of serogroups (*Jensen et al., 2020*).

K. pneumoniae is a type of bacterium that can cause disease under certain conditions, and it is commonly found in the respiratory or intestinal systems of animals. It is a frequent cause of infections that are acquired outside of a hospital setting and poses a significant risk to the health of the public (*Li et al. 2022*). Klebsiella's ability to cause disease is influenced by several factors, including its capsule antigen (which is considered the most significant virulence factor in *K. pneumoniae*), lipopolysaccharide, adhesives, and siderophores (*Struve et al. 2009*).

The bioMérieux VITEK 2 automated system is a very popular tool in clinical microbiology labs for identifying and assessing the susceptibility of bacteria, which includes identifying extended-spectrum beta-lactamases (ESBLs) produced by *E. coli*, *K. pneumoniae* and *K. oxytoca* (*Espinar et al., 2011*).

Materials and methods

1 Samples collection

One hundred samples (100) of Cloacal swab were taken from sick chickens of various ages with watery greenish diarrhea, which were obtained from four separate privately-owned farms in Bilbies,

Sharqiyah Governorate, Egypt. All samples were collected in sterile swabs and transported to Animal Health Research Institute, Ismailia lab. in ice box under complete aseptic conditions as soon as possible for bacteriological examination.

2 Bacteriological examinations

Cloacal swab samples were cultivated aerobically in buffer peptone water, Rapapport Visiladis broth and incubated at 37° C for 18±2 hrs. (*ICMSF,1978; Quinn et al. 2002*) and anaerobically cultivated in a sterile freshly prepared cooked meat medium and incubated anaerobically at 37°C for 24 h in anaerobic jar using gas generating kits (*Smith and Holdeman, 1968*).

A loopful from each sample in the overnight enriched broths were separately streaked into MacConkey's agar medium and incubated at 37°C for 24hr for primary isolation. Colonies were transformed on selective media (EMB, XLD, BGA) and incubated at 37°C for 24hrs. Single colonies were further sub-cultured until pure colonies of bacteria were obtained. The isolated bacteria were identified morphologically (Gram staining) and biochemically using a variety of biochemical reactions. One single colony with a typical colonial appearance and morphological characters was picked up and streaked into semisolid agar medium for preservation and on slope agar

for biochemical and further identification (*Quinn et al. 2002*).

3 Biochemical identifications

Purified suspected isolates were examined by a series of conventional biochemical reactions as follows: Urease test, Utilization of Citrate, Methyl Red reaction (MR), Indole test, Oxidase test, Sugar fermentation test, catalase test, Voges-Proskauer test, Triple sugar iron agar (T.S.I) according to (*Cruickshank, 1968; John et al., 1970; Macfaddin, 1980; Oxoid, 1998; Quinn et al., 2002*)

4 Automated ID&AST

The identification and antibiotic sensitivity of the bacterial isolates were verified automatically using the VITEK 2 Automated ID&AST system, which employs modern fluorescence-based technology. The system comprises of AST and ID-GNB cards, Vitek 2 software, and Advanced Expert System (AES) software from bioMérieux in France, all of which were used in compliance with the manufacturer's instructions (*Ling et al., 2001*).

The process involved sub-culturing the culture strains onto MacConkey agar plates to ensure their purity. The bacterial suspensions were adjusted for turbidity using a densitometer to match the standard of McFarland 0.5 in sterile sodium chloride solution. To prevent changes in turbidity, the suspension preparation time and card filling time was less than 30 minutes. For identification of gram-negative bacilli, the VITEK 2 ID-GNB card was used, which is a

plastic card with 64 wells containing 41 fluorescent biochemical tests (*Funke et al., 1998*), AST card for *Enterobacteriaceae* antimicrobial susceptibility testing (AST) that include the following antimicrobial agents: Cefazolin, Cefepime, Tobramycin, Cefuroxime, Piperacillin, Amikacin, Meropenem, Cefotaxime, Trimethoprim sulfa, Ceftazidime, Levofloxacin, Chloramphenicol, Imipenem, Aztreonam, Cefoxitin, Ciprofloxacin, Gentamycin, Cefoperazone/ sulbactam, Amoxicillin/ CA, Ticarcillin/ CA, Minocycline, Ertapenem, Tigecycline, Moxifloxacin, Ampicillin/sulbactam and bacterial suspension were manually loaded into the VITEK 2 system.

The bacterial suspension was automatically added to each test card, which was then sealed and incubated for 3 hours. The cards were monitored every 15 minutes using kinetic fluorescence measurement, and the VITEK 2 system software analyzed the data and reported the results automatically. The VITEK 2 AST card was used to test the susceptibilities of 14 strains to 25 antimicrobials.

The AES examined the antimicrobial susceptibility data to ensure that the MICs obtained were consistent with the species identification of the organism (*Sanders et al. 2001*) and the recommended susceptibilities interpretations by NCCLS

(*NCCLS,2002*) and The MIC agreement (direct MIC within one twofold dilution of the standard MIC) (*NCCLS.1997*). (*Bruins et al., 2004*).

Standard strains were also included as quality control, and only strains with correct results for all quality control strains were allowed to be tested. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were among the quality control strains used.

Results

1 Bacterial spp. Isolated from samples:

A total of 5 isolates were revealed from the samples which differentiated by different phenotypic methods of identification into *E. coli*, *C. perfringens*, *K. pneumoniae*, *P. mirabilis* & *P.vulgaris* with a percentage of: 39%, 22%14%, 14 % & 11%; respectively and *Salmonellae* spp. can't be found (0%).

2 Morphological characters of *K. pneumoniae* isolates:

The results revealed bright pink mucoid colonies (strong lactose fermenter) on MAC agar and pink mucoid without green metallic sheen on selective EMB medium, yellow colonies / yellow and mucoid colonies on both XLD, BGA medium. The Gram staining of bacterial isolates showed that they are Gram-negative rod-shaped bacillus. These results were confirmed biochemically by different tests. **3 Biochemical**

characters of *K. pneumoniae* isolates: in table 1.

4 Automated ID & AST of *K. pneumoniae* isolates:

Results revealed that the 14 *K. pneumoniae* isolates that had been previously identified by established

method of (Quinn et al. 2002) was rapidly and automatically identified using VITEK 2 system and all isolates were sensitive for 24 different antimicrobial agents as showed in table 2.

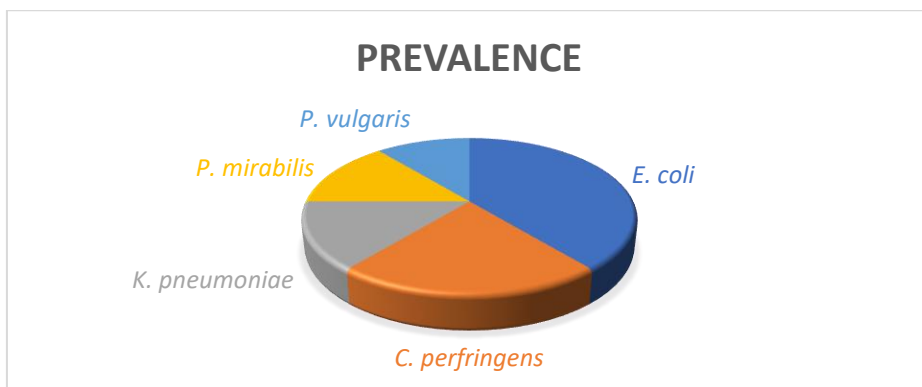


Figure (1): prevalence of isolated microbes

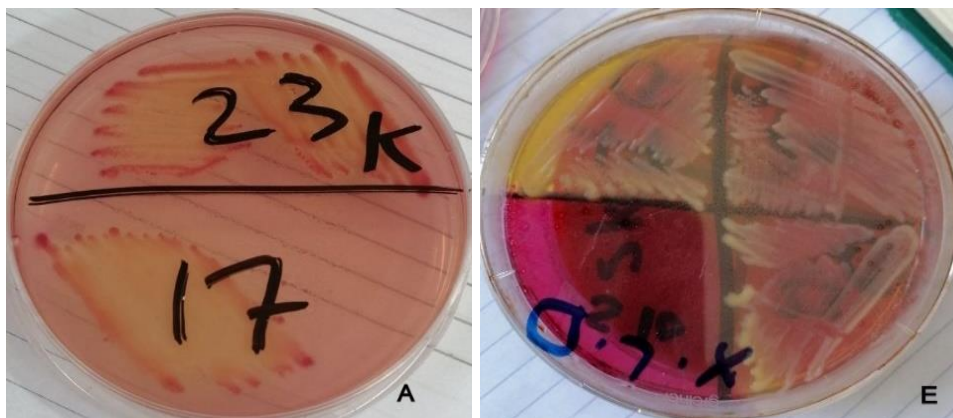


Figure (2): A- *K. pneumoniae* bright pink mucoid colonies on MacConkey's agar

E- *K. pneumoniae* yellow colonies / Yellow and mucoid colonies on XLD medium

Table (1): Biochemical characters of *K. pneumoniae* isolates.

Test	<i>K. pneumoniae</i>	Test	<i>K. pneumoniae</i>
Oxidase	-ve	Indole	-ve
Catalase	+ve	MR (Methyl red)	-ve
Urease	+ve	VP (Voges Proskauer)	+ve
TSI	A/A, + gas, - H ₂ S	Simmons citrate	+ve
Hydrogen sulphide	-ve	Lactose	+ve

*A/A= Acid slant/Acid butt

*K/A= Alkaline slant/Acid butt

*V= Variable

*A/G= Acid and gas

Table (2): results of antimicrobial sensitivity of *K. pneumoniae*

Antimicrobial agents	MIC	R	Antimicrobial agents	MIC	R
Cefazolin	<=2	S	Cefepime	<=0.12	S
Gentamycin	<=1	S	Ciprofloxacin	<=0.015	S
Tobramycin	<=1	S	Cefoperazone/sulbactam	<=16/8	
Piperacillin	<2/4	S	Cefuroxime	<=8	S
Meropenem	<=0.06	S	Amikacin	<=4	S
Trimethoprim sulfa	<=2/38	S	Cefotaxime	<=0.12	S
Levofloxacin	<=0.06	S	Ceftazidime	<=0.5	S
Imipenem	<=0.25	S	Chloramphenicol	<=8	S
Amoxicillin/CA	<=8/4	S	Aztreonam	<=0.25	S
Cefoxitin	<=8	S	Minocycline	<=1	S
Ertapenem	<=0.5	S	Ticarcillin/CA	<=16/2	S
Moxifloxacin	<=0.5	S	Tigecycline	<=0.25	S
Ampicillin/sulbactam	<=8/4	S			

MIC= minimum inhibitory concentration

S= sensitive

Discussion

The chicken industry worldwide is impacted by diverse types of bacteria, such as *K. pneumoniae*, which is a prevalent disease in young chickens and causes significant financial damage. In addition, *K.*

pneumoniae poses a risk to food safety and antibiotic resistance since it could contaminate poultry products such as meat and eggs (Aly *et al.* 2014).

All-over isolation revealed 5 isolates: 39% *E. coli*, 22% *C.*

perfringens, 14% *K. pneumoniae* and 14% *P. mirabilis*, 11% *P. vulgaris* (**Figure 1**) along with no detection of *Salmonellae* (0%). The results of *K. pneumoniae* were nearly similar to that obtained by *El gaos et al. (2019)* and *Dian et al (2020)* who reported its incidence in broiler chicken was 14.4% & 13.75%; respectively and more than that obtained by *Khalda et al. (2000)*, *Abdulrazaq et al. (2014)* and *Aly et al. (2014)*, *Essel et al. (2019)*, who reported that the incidence of *Klebsiella* isolated from chicken was 10.2%, 7%, 10% & 3.5% ;, respectively and less than that obtained by *Younis et al. (2016)*, *Ejikeugwu et al. (2016)* and *Abd-El tawab et al. (2022)* who reported that the incidence of *Klebsiella* isolated from chicken was 15%, 66% & 75%; respectively.

The identification result revealed that *K. pneumoniae* appeared as bright pink mucoid colonies (strong lactose fermenter) on MAC agar and pink mucoid without green metallic sheen on selective EMB medium, yellow colonies / yellow and mucoid colonies on both XLD, BGA medium identified later as *K. pneumoniae* as shown in **Figure (2)**. These results are in agreement with that reported by *Aly et al. (2014)*, *Al-Ammiri et al. (2016)*, *Dian et al. (2020)* and *Abd-Eltawab et al. (2022)*. The Gram staining revealed that *K. pneumoniae* appears as Gram-negative rod-shaped bacillus, these results were in accordance

with that reported by *Aly et al. (2014)*.

The biochemical identification revealed that all *K. pneumoniae* isolates produce an acid butt and slant (yellow/yellow) (A/A) with gas and without H₂S production on TSI and showed negative results in indole, methyl red and positive results in catalase test, citrate, urease and VP. These results agreed with that reported by *Aly et al. (2014)*, *Dian et al. (2020)* and *Abd Eltawab et al. (2022)*.

Antibiotics are extensively used to control infectious disease or as growth promoters in poultry production. Antibiotic treatment is the most important issue that promotes the emergence, spreading and selection of antibiotic resistant microorganisms in both human and veterinary medicine (*Ali et al., 2009*).

Regarding antibiogram of *K. pneumoniae* done by VITEK 2 Automated ID&AST system, previous research has stated that the VITEK 2 Automated ID&AST system is a frequently utilized tool in clinical microbiology labs to determine and recognize bacterial susceptibility profiles (*Espinar et al. 2011*) and that VITEK MS provides cost-effective, fast, and dependable results with high accuracy (*Ling et al. 2001; Luo et al. 2015; Lin et al. 2022*). Numerous investigators employed it to quickly detect *K. pneumoniae* and to evaluate its response to antimicrobial agents as

Daehre et al. (2018), Yang et al. (2019) and Lin et al. (2022).

In the present study, the strain characterization and antimicrobial susceptibility testing of *K. pneumoniae* were performed with the VITEK 2 automated system using the AST and ID-GNB cards, in accordance with the manufacturer's instructions. Results revealed that the 14 *K. pneumoniae* isolates that had been previously identified by established method of (*Quinn et al. 2002*) was rapidly and automatically identified using VITEK 2 system and this agreed with (*Yang et al. 2019*) who reported that all isolated *K. pneumoniae* strains were identified through biochemical tests, staining and the VITEK-2 compact system (bioMerieux, Craponne, France) and also agreed with (*Lin et al. 2022*) who reported that VITEK MS can be used for rapid detection of *K. pneumoniae*.

Also, all isolates were sensitive for 24 different antimicrobial agents: Cefazolin, Cefepime, Ciprofloxacin, Gentamycin, Tobramycin, Cefuroxime, Piperacillin, Amikacin, Meropenem, Cefotaxime, Trimethoprim sulfa, Ceftazidime, Levofloxacin, Chloramphenicol, Imipenem, Aztreonam, Cefoxitin, Amoxicillin/CA, Ticarcillin/CA, Minocycline, Ertapenem, Tigecycline, Moxifloxacin, Ampicillin/sulbactam as showed in **Table (2)**. These results were in line with *Tenover et al. (2006)* who reported that 10 (67%) and 5 (33%) isolates out of 15 was imipenem

susceptible using VITEK and VITEK 2; respectively which indicated that carbapenem resistance in *K. pneumoniae* were not detected by automated susceptibility testing and also in line with *Yin et al. (2021)* who reported that 69.9% of carbapenem-resistant *K. pneumoniae* were susceptible to tigecycline using VITEK 2. Regarding comparison between the current result of *K. pneumoniae* susceptibility using VITEK 2 automated system and results of regular disk diffusion method done by different researchers, the current result agreed with *Aly et al. (2014)* who reported that the sensitivity to Imipenem, Amikacin, Ceftazidime, Meropenem and Ciprofloxacin and also agreed with *Abd Eltawab et al. (2022)* who reported sensitivity to Tobramycin and Amikacin. On the other hand, our results disagreed with that reported by *Fielding et al. (2012); Ojo et al. (2012); Chika et al. (2017); Hayati et al. (2019) and Hermana et al. (2020)* who reported that there was a high level of resistance against many antibiotics. In conclusion, the current research sheds light on the prevalence of various bacteria in chicken farms in Bilbies, Sharqiyah, Egypt, including *E. coli*, *C. perfringens*, *K. pneumoniae* and *Proteus spp.* However, in order to effectively manage outbreaks, it is necessary to identify these bacteria to the species level. This requires more comprehensive and meticulous epidemiology studies. Phenotypic

identification is an important epidemiological tool that can be used to identify *K. pneumoniae*. It is crucial to routinely apply antimicrobial susceptibility testing to select the best antibiotics, especially in cases where multi-drug resistant strains emerge. While the VITEK 2 automated system is a convenient and highly accurate tool, more studies are needed to compare results with the standard method of susceptibility testing.

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التعرف الآلي واختبار الحساسية للمضادات الميكروبية باستخدام VITEK 2 وانتشار
K.pneumoniae المعزولة من الدجاج المصاب بالإسهال في محافظة الشرقية
 أحمد ر. خفاجي¹، ندى عيروس¹، وليد ف. خليل²، فاطمة يوسف³، منى أحمد⁴، فاطمة
 الزهراء محمد⁵

1 قسم البكتيريا والمناعة وعلم الفطريات بكلية الطب البيطري، جامعة قناة السويس، الإسماعيلية،
 41522، مصر

2 قسم علم الأدوية بكلية الطب البيطري، جامعة قناة السويس، الإسماعيلية، 41522، مصر
 3- قسم الباثولوجيا بمعهد بحوث صحة الحيوان بالإسماعيلية.

4- قسم علم البكتريولوجي بمعهد بحوث صحة الحيوان بالإسماعيلية.

5 طبيب بيطري حر

الملخص العربي:

تم اجراء هذه الدراسة لعزل وتصنيف بعض المسببات البكتيرية للإسهال والتي تصيب الدواجن حيث
 تم تجميع 100 مسحة من الدواجن التي تم تربيتها في 4 مزارع خاصة في بلبس محافظة الشرقية وتم
 إجراء الفحص البكتيري الخاص بهذه العينات لمعرفة المسبب الاساسي للإسهال. وقد أوضحت النتائج
 وجود 5 عزلات 39% الايشيرشيا الكولونية، 22% كلوستريديم برفرنجيز، 14% كلبسيلا نيمونيا،
 14% بروتيس ميربلز، 11% بروتيس فولجارز ولم يتم تحديد اية معزولات للسالمونيل
 تم التعرف علي جميع معزولات الكلبيسيلا نيمونيا باستخدام جهاز الفيتك واتضح ان كل المعزولات
 حساسة ل24 نوع من المضادات الحيوية والتي تشمل كلا من السيفازولين السيفابم السيبروفلوكساسين
 الجنتاميثين والتوبراميسين السيفوكسيم البيراسيلين الاميكاسين الميروبنين السيفاتوكسيم الترميسوبريم
 سلفا السيفتازيديم الليفوفلوكساسين الكلورامفينيكول الامبينيم الازوتينام السيفاتوكسين الاموكساسلين
 التايكارسيلين المينوسيكلين الايتراينيم والتيجيسيكلسن المكسيفلوكساسين والامبيسلين