CASES OF MULTI DRUG RESISTANCE (MDR) IN KLEBSIELLA PNEUMONIAE ISOLATED FROM HEALTHY PIGS

by Hani Plumeriastuti

Submission date: 15-Jun-2023 05:00PM (UTC+0800)
Submission ID: 2116524236
File name: ance_MDR_in_Klebsiella_pneumoniae_Isolated_from_Healthy_Pigs.pdf (152.54K)
Word count: 5113
Character count: 27460

Biochem. Cell. Arch. Vol. 21, Supplement 1, pp. 1979-1985, 2021 | www.connectjournals.com/bca | *ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2021.21.1979 | *eISSN 0976-1772*

CASES OF MULTIDRUG RESISTANCE (MDR) IN *KLEBSIELLA PNEUMONIAE* ISOLATED FROM HEALTHY PIGS

Eka Dian Sofiana[1], Mustofa Helmi Effendi[2]*, Hani Plumeriastuti[3] and Junianto Wika Adi Pratama[4]

[1]Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Raya Mulyorejo, Surabaya 60115, East Java, Indonesia.
[2]Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Raya Mulyorejo, Surabaya 60115, East Java, Indonesia.
[3]Division of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Raya Mulyorejo, Surabaya 60115, East Java, Indonesia.
[4]Department of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University. Jl. Dukuh Kupang XXV No.54, Surabaya 60225
*e-mail: mheffendi@yahoo.com

(Received 12 May 2021, Revised 2 August 2021, Accepted 11 August 2021)

ABSTRACT : Antibiotics are commonly used in veterinary medicine throughout the world for therapeutic uses and for increasing production in pig farms. *Klebsiella pneumoniae* is one of the most important organisms clinically that has received attention in public health. *Klebsiella pneumoniae* (*K. pneumoniae*) is a group of Enterobacteriaceae which is significant for causing disease and shows frequent resistance to antibiotics in humans as well as in pigs. This study aims to determine the antibiotic resistance profile in pig farms through the identification of *K. pneumoniae*, which is one of the important bacteria involved in antibiotic resistance. This study focuses on the presence of *K. pneumoniae* bacteria in pigs carried out by rectal swabs on two pig farms in East Java, namely the pig farms in Gresik Regency and Malang Regency. The samples obtained were cultured using Mac Conkey Agar media and tested for biochemical identification and antibiotic sensitivity testing with the Kirby-Bauer method against the antibiotics ciprofloxacin, streptomycin, trimethoprim, tetracyclin and aztreonam. The *K. pneumoniae* bacteria was isolated from 7 samples of swine rectal swabs from pig farms in Gresik Regency and 4 samples from pig farms in Malang Regency. Of the 11 positive samples of *K. pneumoniae*, almost all isolates were resistant to tetracyclin and trimethoprim antibiotics. There were 4 *K. pneumoniae* isolates that were resistant to 3 antibiotics (MDR). It can be concluded that *K. pneumoniae* has potential to become a serious problem on public health.

Key words : Antibiotic resistance, *Klebsiella pneumoniae*, multidrug resistance, pigs, public health.

How to cite : Eka Dian Sofiana, Mustofa Helmi Effendi, Hani Plumeriastuti and Junianto Wika Adi Pratama (2021) Cases of multidrug resistance (MDR) in *Klebsiella pneumoniae* isolated from healthy pigs. *Biochem. Cell. Arch.* **21**, 1979-1985. DocID: https://connectjournals.com/03896.2021.21.1979

INTRODUCTION

Antibiotic resistance cases have increased significantly, indicating a mortality rate of up to 50,000 people each year (WHO, 2017). Problems associated with the development and spread of antibiotic resistance are major health problems that are spreading rapidly around the world and are currently seen as a major threat to public health at a global level (Riwu *et al*, 2020). The widespread and inappropriate use of antibiotics has resulted in the emergence of strains of bacteria that are resistant to antibiotics (Widodo *et al*, 2020).

Antibiotics have been commonly used in veterinary medicine throughout the world for therapeutic uses and to increase production in livestock (Widodo *et al*, 2020; Khairullah *et al*, 2020). The use of antibiotics in pig farms in Indonesia is still widely practiced by pig breeders themselves, where the level of farmer knowledge about antibiotics and antibiotic resistance is generally still low. Many farmers determine their own use of antibiotics on their farms based on their own experience and input from other breeders (Arief *et al*, 2016). Therefore, 90% of the digested dose can be excreted unmodified or partly metabolized directly through urine and feces. As a result, the feces of pigs given antibiotic treatment becomes an important reservoir in terms of antibiotic residues and bacteria that are resistant to many classes of antibiotics or multidrug resistance (MDR) (Jury *et al*, 2010;

Hidayatullah *et al*, 2020).

Bacteria that have MDR properties will be more difficult and take longer to treat, and may even require new antibiotics as treatment (WHO, 2017). It has been reported in the World Health Organization's (WHO) global surveillance of bacterial resistance to antibiotics, *Klebsiella pneumoniae* (*K. pneumoniae*) is classified as one of nine bacteria involved in antibiotic resistance. *K. pneumoniae* is one of the most important organisms clinically that has received attention in public health (Effendi *et al*, 2018). *K. pneumoniae* is a group of Enterobacteriaceae which is significantly considered as an opportunistic pathogen as a cause of disease and shows frequent resistance to antibiotics (Effah *et al*, 2020).

K. pneumoniae becomes resistant to several classes of antibiotics because it can produce beta-lactamase enzymes, which can deactivate the effectiveness of the antibiotics. There have been many *K. pneumoniae* that is resistant to several classes of antibiotics or multidrug resistance (MDR) in pig farms in European and Asian countries, but there are no reports of MDR incidence in pig farms in Indonesia (Sofiana *et al*, 2020). *K. pneumoniae* is known to be pathogenic and cause respiratory problems in pigs, which can lead to death (Bidewell *et al*, 2018). *K. pneumoniae* bacteria detected in animals are increasing, this allows the beta-lactamase-producing *K. pneumoniae* to contribute to the increased incidence of infection with beta-lactamase-producing bacteria in humans (Effendi *et al*, 2018; Mobasseri *et al*, 2019).

The high pig population in Indonesia and the lack of application of biosafety and biosecurity in the pig farming sector are among the factors for the spread of resistant bacteria (Arief *et al*, 2016). Animals spread bacteria that are resistant to antibiotics through feces. Resistant bacteria contained in feces can re-enter the human population through direct contact between animals and humans or vice versa, through water, food and the surrounding environment (Ansharieta *et al*, 2020; Widodo *et al*, 2020). Pigs are agents of the spread of *K. pneumoniae* bacteria which have MDR properties to other animals, the environment and humans (Yang *et al*, 2019; Sofiana *et al*, 2020).

Today awareness of human and animal health is inseparably linked to their environment leading to an integrated One Health approach, especially focusing on food safety, zoonotic surveillance and antibiotic resistance control (Rahmahani *et al*, 2020; Effendi *et al*, 2021). The discovery of many *K. pneumoniae* that is resistant to antibiotics in animals has a negative impact on public health and has an impact on the economy of a country, so there needs to be a control or prevention so that this problem can be resolved (Permatasari *et al*, 2020). Therefore, study aims to determine the antibiotic resistance profile in pig farms through the identification of *K. pneumoniae*, which is one of the important bacteria involved in antibiotic resistance. This study focuses on the presence of *K. pneumoniae* bacteria in pigs carried out by rectal swabs on two pig farms in East Java, namely the pig farms in Gresik Regency and Malang Regency.

MATERIALS AND METHODS

Sample collection and preparation

In this study, a total sample of 130 rectal swabs were obtained from 2 locations in East Java. Sampling was carried out using transport medium of amies viscosa, swab was carried out aseptically on pigs in Gresik Regency farms as many as 80 samples and 50 samples in farms in Malang Regency. The transportation process of all the rectal swab sample specimens obtained were put into a cool box which also equipped with an cool pack (Effendi *et al*, 2019). A total of 130 samples were cultured using inoculating loop and on MCA media then incubated at 37^0C for 24 hours (Wibisono *et al*, 2020; Permatasari *et al*, 2020).

Characterization of isolates

Pure bacterial isolates were identified based on colony morphology, cell morphology and biochemical tests. Colony morphology observations were seen from the shape, color and edges of bacterial colonies on MCA media. Observation of cell morphology includes the shape and arrangement of cells selected through Gram stain. Furthermore, each isolate was characterized biochemically. Biochemical tests were carried out to see the characteristics of Klebsiella pneumoniae bacteria through biochemical reactions on Simon's Citrate Agar, Semi Solid Agar media. MR-VP media, Kligler's Iron Agar, peptone water which is then dropped by kovach reagent for Indol test and urea media (Leber, 2016; Permatasari *et al*, 2020).

Antibiotic sensitivity test

Antibiotic sensitivity test was performed using the Kirby-Baurer agar diffusion method. The antibiotics used are those that are already on the disc. The clear zone formed is then grouped into sensitive (S), intermediate (I) or resistant (R) groups. The procedure in the antibiotic sensitivity test is first of all the bacteria culture obtained from the colony contained in the MCA media dissolved in a test tube containing 8 ml of physiological NaCl, homogenized using a vortex until turbidity is obtained

which is the same as the Mc Farland standard of 0.5 (Putra *et al*, 2019). Physiological nacl that has been tested for its recurrence with the Mc Farland standard of 0.5 is then applied to a sterile cotton swab and then gently rubbed on the entire surface of the Mueller Hinton Agar medium (Putra *et al*, 2020; Wibisono *et al*, 2020). Sensitivity test and resistance profiling were carried out using 5 types of antibiotics, namely ciprofloxacin 5 µg, streptomycin 10 µg, tetracyclin 30 µg, trimethoprim 5 µg and aztreonam 30 µg. Bacterial cultures were incubated at 35-37°C for 18-24 hours (CLSI, 2018).

After the test results were obtained, the bacteria were grouped into the Multi drug Resistance (MDR) or non MDR groups. MDR bacteria are bacteria that are resistant to 3 or more classes of antibiotics (Magiorakos *et al*, 2012; Harijani *et al*, 2020).

RESULTS AND DISCUSSION

The results showed 11 out of 130 samples isolated from the pig rectal swab were positive for *K. pneumoniae*. The results of colony morphological identification on Mac Conkey Agar media are shown in Fig. 1 and the results of biochemical tests will be presented in Fig. 2. The percentage of swab swab swab samples from swab rectals after identification and biochemical tests obtained positive samples of *K. pneumoniae* bacteria as much as 8.75% (7 / 80) samples from pig farms in Gresik Regency and 8% (4/50) samples from pig farms in Malang Regency, so that the total number of positive samples for *K. pneumoniae* bacteria was 8.5% (11/130) samples which is shown in Table 1.

The level of bacterial resistance to antibiotics is obtained by measuring the diameter of the inhibition zone formed after the antibiotic disc attachment process. The standard for assessing the diameter of the inhibition zone for antibiotics based on the CLSI (Clinical Laboratory Standards Institute) is a reference for comparing the measurement results of the inhibition zone obtained in this study. In this study, there were 5 types of antibiotic discs used, namely ciprofloxacin (5 µg), streptomycin (10 µg), tetracyclin (30 µg), trimethoprim (5 µg) and aztreonam (30 µg). The results of the inhibition zone of the antibiotics formed on MHA media were then measured using a caliper (mm) in diameter. The results of antibiotic resistance tests are shown in Fig. 3.

Fig. 1 : Isolation of *K. pneumoniae* bacterial colonies on Mac Conkey Agar (MCA) selective media. **Note:** *K. pneumoniae* bacteria culture on MCA media looks pink, round in shape, the surface of the colony looks smooth and moist.

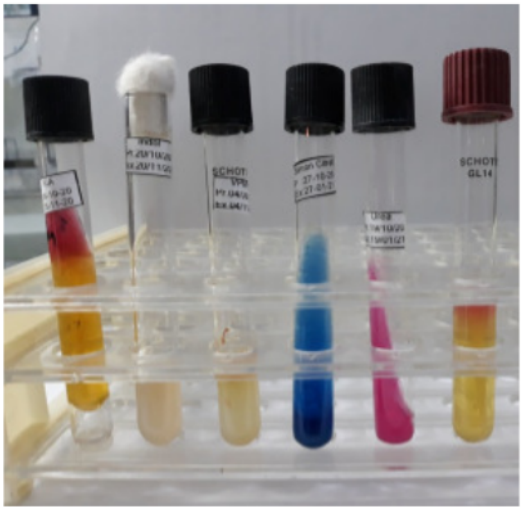

Fig. 2 : Biochemical test results of bacterial isolates identified as *Klebsiella pneumoniae*. **Note:** From left to right are biochemical media: KIA, Indol, MR-VP, Simon Citrat, Urea and Semi Solid Media.

The results of the antibiotic resistance test in this study were indicated by the presence of an antibiotic inhibition zone against bacterial growth. In this study, 91%

Table 1 : Number of positive samples for *Klebsiella pneumoniae* bacteria.

Location	Sample size	Positive samples	Sample Code
Gresik Farm	80	7	GB24, GB29, GB30, GB32, GB33, GB37, GB41
Malang Farm	50	4	MB60, MB81, MB82, MB91

Note: GB (Gresik) followed by the sample number and MB (Malang) followed by the sample number.

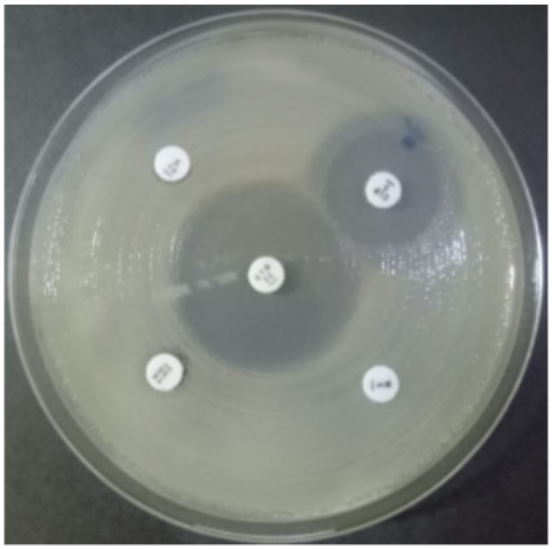

Fig. 3 : Antibiotic resistance test results against *K. pneumoniae*. **Note :** CIP (ciprofloxacin 5 µg), S (streptomycin 10 µg), TE (tetracyclin 30 µg), W (trimethoprim 5 µg) and ATM (aztreonam 30 µg).

re Multidrug Resistance (MDR) bacteria. MDR is the incidence of antibiotic resistance in isolates that are resistant to ≥3 types of antibiotics (Magiorakos *et al*, 2012; Permatasari *et al*, 2020). The incidence of MDR in *K. pneumoniae* bacteria resulted in treatment being longer and difficult to cure. By looking at the pattern of antibiotic resistance, it is hoped that it can be a guide in choosing the right antibiotic for treatment (Hayati *et al*, 2019).

This study also showed that the bacterial isolates found were still sensitive to the antibiotics Ciprofloxacin and Aztreonam and some were still intermediates to Streptomycin. Bacterial resistance to antibiotics occurs due to inappropriate and excessive use of antibiotics. *K. pneumoniae* resistance to antibiotics occur because these bacteria have the ability to produce Extended Spectrum Beta Lactamase (ESBL). Beta-lactamase zymes can protect Gram-negative bacteria against beta-lactam antibiotics. The target of beta-lactam antibotic attack is the cell wall. Antibiotics in this class have beta-lactam groups as well as cell walls that react with enzymes

Table 2 : Pattern of antibiotic resistance to the *Klebsiella pneumoniae*.

No	Antibiotics	Sample code										
		GB24	GB29	GB30	GB32	GB33	GB37	GB41	MB60	MB81	MB82	MB91
1	Ciprofloxacin	S (22)	S (21)	S (27)	S (30)	S (23)	S (27)	S (21)	S (30)	S (29)	S (30)	S (22)
2	Streptomycin	R (6)	R (5)	I (12)	I (14)	I (12)	I (13)	R (5)	R (11)	I (12)	I (13)	R (6)
3	Tetracyclin	R (6)	R (7)	R (8)	R (10)	R (6)	R (9)	R (5)	I (14)	R (6)	R (9)	R (6)
4	Trimethoprim	R (5)	R (5)	R (8)	R (10)	R (6)	R (6)	R (5)	S (26)	R (6)	R (6)	R (6)
5	Aztreonam	S (30)	S (29)	S (31)	S (31)	S (30)	S (30)	S (29)	S (32)	S (31)	S (34)	S (30)
Total	3	3	2	2	2	2	3	1	2	2	3	

Note: R (resistance), I (intermediate), S (sensitive), the number in brackets is the diameter of the drag zone in mm.

(10/11) of samples were resistant to Tetrcyclin and Trimethoprim, namely the samples GB24, GB29, GB30, GB32, GB33, GB37, GB41, MB 81, MB82 and MB91 with a zone size of ≤ 11 mm. Antibiotic resistance testing on all positive isolates of *K. pneumoniae* showed that 4 isolates were resistant to 3 antibiotics and can be classes as Multidrug resistance (MDR). The results of the antibiotic inhibition zone measurements is presented in the Table 2.

DISCUSSION

The results of biochemical identification showed that the *K. pneumoniae* bacteria had a positive Simon's Citrate character, a negative or non-motile motility test, negative Methyl Red, positive Voges Proskauer, the KIA test on the slant/ slant was acidic and at the bottom was acidic and produced gas but H_2S negative, negative indole and positive urea (Leber, 2016). The results showed that there were 36% (4/11) of *K. pneumoniae* isolates which in the process of making cell walls (Ansharieta *et al*, 2021; Wibisono *et al*, 2021). Enzymes will no longer function so that the cell walls will not form completely. Cell walls that are not fully formed and bacterial cells without cell wall cause bacteria to die (Finley *et al*, 2013; Kristianingtyas *et al*, 2020).

The widespread use of antibiotics and without close supervision has led to the emergence of resistance to antibiotics. The majority of pig farms in Indonesia use antibiotics for both treatment and prevention (Arief *et al*, 2016; Faridah *et al*, 2020). As many as 82% of *K. pneumoniae* isolates were resistant to tetracycline and trimethoprim in this study. Tetracyclines and trimethoprim are the antibiotics that are most widely used by livestock in Indonesia and around the world because of their efficacy as broad spectrum antibiotics, easy to absorb, low prices and low side effects (Arief *et al*, 2016).

Tetracyclin antibiotics in certain countries are even

commonly used as additional feed ingredients in pig farms. Tetracyclin is well absorbed and has low toxicity (Michalova *et al*, 2004). This is also supported by research conducted in Malaysia, *Klebsiella pneumoniae* in pigs was found to be resistant to several antibiotics including: ciprofloxacin, aztreonam, ampicillin, tazobactam, amikacin, tetracyclin. In that study, the highest level of antimicrobial resistance to tetracyclin antibiotics. All strains from the agricultural environment and pigs show resistance to tetracyclin, which is widely used in feed supplements (HAIAP, 2013). Research conducted by Yang in China also revealed that the *Klebsiella pneumoniae* bacteria in pigs was 74.5% resistant to tetracyclin (Yang *et al*, 2019). In Kieffer's study, it was stated that swab rectals of pigs on Portuguese farms tested positive for *K. pneumoniae*, which was resistant to tetracyclin and trimethoprim (Kieffer *et al*, 2017). In Founou's study in Cameroon, ESBL-producing *K. pneumoniae* in pigs was resistant to trimethoprim. This study also reported that all *K. pneumoniae* in pigs and humans showed reduced susceptibility to trimethoprim (Founou *et al*, 2018).

The results of this study indicated that 55% (6/11) of the isolates had an intermediate inhibition zone to streptomycin and 45% (5/11) of the isolates were resistant to streptomycin. The mechanisms of chromosomal resistance to aminoglycosides in *K. pneumoniae* include modification of cell permeability due to changes in the efflux pump system and due to loss of putative porin (KpnO). This may indicate a different affinity of the permeability apparatus with different aminoglycosides. Direct involvement in aminoglycoside resistance was reported in vitro for missing porin KpnO leading to resistance to tobramycin, streptomycin and spectinomycin (Amador *et al*, 2019).

Antibiotics that are often used in pig farms besides tetracyclines are the fluoroquinolones which are widely used in humans and animals as a therapy for digestive and respiratory disorders (Arief *et al*, 2016). Of the 11 *K. pneumoniae* isolates tested, all isolates still showed sensitivity to ciprofloxacin. Since 1998, fluoroquinolones have been classified by WHO as critically important in human medicine because of their importance in treating *Campylobacter*, *Salmonella* and *E. coli* infections. To prevent further resistance, fluoroquinolone treatment is limited to individual, not group treatment. Even in European countries, the use of this antibiotic has been banned for use on farms (Hayati *et al*, 2019).

Another β-lactam group, treonam, is not used in pig farms, but is commonly used in the treatment of bacterial infections in humans, especially in cases of infections that are resistant to ampicillin and amoxicillin. Resistance of this type of antibiotic can make treatment in humans difficult and takes longer. Bacteria resistant to aztreonam indicated ESBL-producing bacteria, but the results of this test had low sensitivity. Research conducted by Sanguinetti, showed that as many as 58% of bacterial isolates declared as non-ESBL bacteria had ESBL coding genes. This biased result could be due to the fact that ESBL bacteria can go undetected or the bacteria produce other enzymes that hydrolyze other β-lactam antibiotics such as cefpodoxime and cefepim (Sanguinetti *et al*, 2003).

The sensitivity of the test can be increased by using another method, namely the Double Disc Diffusion (DDD) method which combines cefpodoxime (10µg) and clavulanic acid or also using the Phoenix ESBL test. In addition, ESBL bacteria can be identified by detecting the presence of the ESBL coding gene. If the coding gene is detected in a bacterial isolate that is still sensitive on the antibiotic sensitivity test, then the isolate is considered a resistant isolate (CLSI, 2018). In order to prevent the further spread of antibiotic resistant bacteria, it is hoped that breeders can apply good hygiene and sanitation in the enclosure environment and choose a veterinarian or paramedic as a person, who is considered an expert and is authorized to take medical measures for livestock (Sofiana *et al*, 2020). The isolation of this *K. pneumoniae* strain always urges the application of strict infection and control measures and constant surveillance of antibiotic resistance in the hospital. Similar rigorous interventions must be made in the food production industry if we are to successfully prevent in the spread of their clones in the food chain from livestock to the dining table (Founou *et al*, 2016; Hartadi *et al*, 2020).

In conclusion can be showed that *K. pneumoniae* bacteria was successfully isolated from swab samples of swine in 2 pig farms in East Java, namely Gresik Regency and Malang Regency by 8.5% (11/130). Almost all isolates showed resistance to tetracyclin and trimethprim, amoxicillin 91% (10/11). There were 36% (4/11) four isolates out of eleven positive isolates of *K. pneumoniae* bacteria that were isolated were resistant to 3 classes of antibiotics or Multidrug Resistance (MDR). MDR in *K. pneumoniae* is becoming a serious problem in humans and animals, increasing resistance to most of the available antibiotics and causing treatment difficulties. Farmers are expected to be more vigilant and need to apply biosafety and biosecurity to prevent further spread of these antibioic resistant bacteria in animals, livestock environments, slaughterhouses, surrounding environments and humans.

ACKNOWLEDGEMENTS

This research was in part funded by the Direktorat Riset dan Pengabdian Masyarakat, Deputi Bidang Penguatan Riset dan Pengembangan Kementerian Riset dan Teknologi/ Badan Riset dan Inovasi Nasional, Indonesia in fiscal year 2020 with grant number : 756/ UN3.14/PT/2020.

REFERENCES

Amador P, Fernandes R, Prudencio C and Duarte I (2019) Prevalence of Antibiotic Resistance Genes in Multidrug-Resistant Enterobacteriaceae on Portuguese Livestock Manure. *Antibiotics* **8**(1), 23.

Ansharieta R, Effendi M H and Plumeriastuti H (2020) Detection of Multidrug-Resistant (MDR) *Escherichia coli* isolated from Raw Milk in East Java Province, Indonesia. *Indian J. Forensic Med. & Toxicol.* **14**(4), 4303-4307.

Ansharieta R, Effendi M H, Ramandinianto S C and Plumeriastuti H (2021) Molecular Identification of bla_{CTX-M} and bla_{TEM} Genes Encoding Extended Spectrum β-Lactamase (ESBL) Producing *Escherichia coli* Isolated from Raw Cow's Milk in East Java, Indonesia. *Biodiversitas* **22** (4), 1600-1605.

Arief R A, Darmawan R D, Sunandar M D W, Widyastuti E, Nugroho A, Jatikusumah A A G, Putra E, Basuno A, Karuniawati A, Suwandono I, Willyanto I and Suandy H Latif (2016) Penggunaan Antibiotik pada Peternakan Babi di Provinsi Jawa Tengah, Indonesia. Prosiding KIVNAS ke-14, ICE-BSD City, Tangerang, pp 161-163.

Bidewell C A, Williamson S M, Rogers J, Tang Y, Ellis R J, Petrovska L and AbuOun M (2018) Emergence of *Klebsiella pneumoniae* subspecies pneumoniae as a cause of septicaemia in pigs in England. *PLoS One* **13**(2), e0191958.

Clinical and Laboratory Standards Institute (CLSI) (2018) *Performance Standards for Antimicrobial Susceptibility Testing*. 28th Edition. Clinical and Laboratory Standards Institute.

Effah C Y, Sun T, Liu S and Wu Y (2020) *Klebsiella pneumoniae*: an increasing threat to public health. *Ann. Clin. Microbiol. Antimicrob.* **19**(1), 1.

Effendi M H, Bintari I G, Aksono E B and Hermawan I P (2018) Detection of *bla*TEM gene of *Klebsiella pneumoniae* isolated from Swab of Food Producing Animals in East Java. *Trop. Animal Sci. J.* **41**(3), 174-178.

Effendi M H, Harijani N, Budiarto, Triningtya N P, Tyasningsih W and Plumeriastuti H (2019) Prevalence of Pathogenic *Escherichia coli* Isolated from Subclinical Mastitis in East Java Province, Indonesia. *Indian Vet. J.* **96** (03), 22–25.

Effendi M H, Tyasningsih W, Yurianti Y A, Rahmahani J, Harijani N and Plumeriastuti H (2021) Presence of multidrug resistance (MDR) and extended -spectrum beta-lactamase (ESBL) of *Escherichia coli* isolated from cloacal swabs of broilers in several wet markets in Surabaya, Indonesia. *Biodiversitas* **22** (1), 304-310.

Faridah H D, Dewi E K, Fatimah Effendi M H and Plumeriastuti H (2020) A Review of Antimicrobial Resistance (AMR) of *Escherichia coli* on Livestock and Animal Products: Public Health Importance. *Sys. Rev. Pharm.* **11**(11), 1210-1218.

Finley R L, Collignon P, Larsson D G, McEwen S A, Li X Z, Gaze W H, Reid-Smith R, Timinouni M, Graham D W and Topp E (2013) The scourge of antibiotic resistance: the important role of the environment. *Clin. Infect. Dis.* **57**(5), 704-710.

Founou L L, Founou R C, Allam M, Ismail A, Djoko C F and Essack S Y (2018) Genome Sequencing of Extended-Spectrum β-Lactamase (ESBL)-Producing *Klebsiella pneumoniae* Isolated from Pigs and Abattoir Workers in Cameroon. *Front. Microbiol* **9**, 188.

Founou L L, Founou R C and Essack S Y (2016) Antibiotic Resistance in the Food Chain: A Developing country-Perspective. *Front. Microbiol.* **7**, 1881.

Harijani N, Oetama S J T, Soepranianondo K, Effendi M H and Tyasningsih W (2020) Biological Hazard on Multidrug Resistance (MDR) of *Escherichia coli* Collected From Cloacal Swab of Broiler Chicken on Wet Markets Surabaya. *Indian J. Forensic Med. Toxicol.* **14** (4), 3239-3244.

Hartadi E B, Effendi M H, Plumeriastuti H, Sofiana E D, Wibisono F M and Hidayatullah A R (2020) A Review of Enterotoxigenic *Escherichia coli* Infection in Piglets: Public Health Importance. *Syst. Rev. Pharm.* **11**(9), 687-698.

Hayati M, Indrawati A, Mayasari N, Istiyaningsih I and Atikah N (2019) Molecular detection of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates of chicken origin from East Java, Indonesia. *Vet. World* **12**(4), 578–583.

Health Action International Asia Pacific (HAIAP) (2013) Third World Network (TWN) Penang in association with Consumers' Association of Penang. (2013). Antibiotic Use and Antibiotic Resistance in Food Animals in Malaysia: A Threat to Human and Animal Health.

Hidayatullah A R, Effendi M H, Plumeriastuti H, Wibisono F M, Hartadi E B and Sofiana E D (2020) A Review of the Opportunistic Pathogen *Citrobacter freundii* in Piglets Post Weaning : Public Health Importance. *Sys. Rev. Pharm.* **11**(9), 767-773.

Jury K L, Vancov T, Stuetz R M and Khan S J (2010) Antibiotic resistance dissemination and sewage treatment plants. In : *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* (Méndez-Vilas A ed.). Formatex Research Center: Badajoz, Spain. pp. 509–519. ISBN 978-84-614-6194-3.

Khairullah A R, Ramandinianto S C and Effendi M H (2020) A Review of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) on Bovine Mastitis. *Sys. Rev. Pharm.* **11**(7), 172-183.

Kieffer N, Aires-de-Sousa M, Nordmann P amd Poirel L (2017) High Rate of MCR-1–Producing *Escherichia coli* and *Klebsiella pneumoniae* among Pigs, Portugal. *Emerg. Infect. Dis.* **23**(12), 2023-2029.

Kristianingtyas L, Effendi M H, Tyasningsih W amd Kurniawan F (2020) Genetic Identification of blactx-M Gene and blatem Gene on Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli* from Dogs. *Indian Vet. J.* **97** (01), 17 – 21.

Leber A L (2016) 4th edition. *Clinical Microbiology Procedures Handbook.* Vol. 1, American Society for Microbiology Press, Washington DC.

Magiorakos A P, Srinivasan A, Carey R B, Carmeli Y, Falagas M E, Giske C G, Harbarth S, Hindler J F, Kahlmeter G, Olsson-Liljequist B, Paterson D L, Rice L B, Stelling J, Struelens M J, Vatopoulos A, Weber J T and Monnet D L (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant

bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **18**, 268-281.

Michalova E, Novotna P and Schlegelova J (2004) Tetracyclines in veterinary medicine and bacterial resistance to them. *Vet. Med. Czech*. **49**(3), 79-100.

Mobasseri G, The S J, Ooi P T, Shiang C T and Thong K L (2019) Molecular characterization of multidrug-resistant and extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolated from Swine farms in Malaysia. *Microbial Drug Resistance* **25**(7), 1087-1098

Permatasari D A, Witaningrum A M, Wibisono F J and Effendi M H (2020) Detection and prevalence of multidrug-resistant *Klebsiella pneumoniae* strains isolated from poultry farms in Blitar, Indonesia. *Biodiversitas* **21** (10), 4642-4647.

Putra A R, Effendi M H, Koesdarto S, Suwarno S, Tyasningsih W and Estoepangestie A T (2020) Detection of the extended spectrum â-lactamase produced by *Escherichia coli* from dairy cows by using the Vitek-2 method in Tulungagung regency, Indonesia. *Iraqi J. Vet. Sci*. **34** (1), 203-207.

Putra A R S, Effendi M H, Koesdarto S and Tyasningsih W (2019) Molecular identification of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* isolated from dairy cows in East Java Province, Indonesia. *Indian Vet. J.* **96** (10), 26 – 30.

Rahmahani J, Salamah, Mufasirin, Tyasningsih W and Effendi M H (2020) Antimicrobial resistance profile of *Escherichia coli* from cloacal swab of domestic chicken in Surabaya traditional market. *Biochem. Cell. Arch*. **20** (1), 2993-2997.

Riwu K H P, Effendi M H and Rantam F A (2020) A Review of Extended Spectrum β-Lactamase (ESBL) producing *Klebsiella pneumoniae* and Multidrug Resistant (MDR) on Companion Animals. *Sys. Rev. Pharm.* **11**(7), 270-277.

Sanguinetti M, Posteraro B, Spanu T, Ciccagliano D, Romano L, Fiori B, Nicolletti G, Zanetti S and Fadda G (2003) Characterization of clinical isolates of *Enterobacteriaceae* from Italy by the BD phoenix extended-spectrum β-lactamase detecion method. *JCM* **41**(4), 1463-1468.

Schmithausen R M, Schulze-Geisthoevel S V, Heinemann C, Bierbaum G, Exner M, Petersen B and Steinhoff-Wagner J (2018) Reservoirs and transmission pathways of resistant indicator bacteria in the Biotope pig stable and along the food chain: A review from one health perspective. *Sustainability* **10**, 3967.

Sofiana E D, Pratama J W A, Effendi M H, Plumeriastuti H, Wibisono F M and Hartadi E B (2020) A review of the presence of antibiotic resistance problems on *Klebsiella pneumoniae* acquired from pigs: public health importance. *Sys. Rev. Pharm.* **11**(9), 535-543.

Wibisono F J, Sumiarto B, Untari T, Effendi M H, Permatasari D A and Witaningrum A M (2020) CTX gene of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* on broilers in Blitar, Indonesia. *Sys. Rev. Pharm.* **11**(7), 396-403.

Wibisono F J, Sumiarto B, Untari T, Effendi M H, Permatasari D A and Witaningrum A M (2020) The presence of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* on layer chicken farms in Blitar Area, Indonesia. *Biodiversitas* **21** (6), 2667-2671.

Wibisono F J, Sumiarto B, Untari T, Effendi M H, Permatasari D A and Witaningrum A M (2020) Pattern of antibiotic resistance on extended-spectrum beta-lactamases genes producing *Escherichia coli* on laying hens in Blitar, Indonesia. *Biodiversitas* **21** (10), 4631- 4635.

Wibisono F J, Sumiarto B, Untari T, Effendi M H, Permatasari D A and Witaningrum A M (2021) Molecular identification of CTX gene of extended spectrum beta-lactamases (ESBL) producing *Escherichia coli* on layer chicken in Blitar, Indonesia. *The J. Anim. Plant Sci.* **31** (4), 954-959.

Widodo A, Effendi M H and Khairullah A R (2020) Extended-spectrum beta-lactamase (ESBL)-producing *Eschericia coli* from livestock. *Sys. Rev. Pharm*. **11**(7), 382-392.

Yang F, Deng B, Liao W, Wang P, Chen P and Wei J (2019) High rate of multiresistant *Klebsiella pneumoniae* from human and animal origin. *Infect. Drug Resist.* **12**, 2729-2737.

CASES OF MULTI DRUG RESISTANCE (MDR) IN KLEBSIELLA PNEUMONIAE ISOLATED FROM HEALTHY PIGS

ORIGINALITY REPORT

19%	16%	15%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.frontiersin.org Internet Source	1%
2	academic.oup.com Internet Source	1%
3	www.thejaps.org.pk Internet Source	1%
4	e-jmsb.id Internet Source	1%
5	core.ac.uk Internet Source	1%
6	f1000research.com Internet Source	1%
7	mdpi-res.com Internet Source	1%
8	repository.nwu.ac.za Internet Source	1%
9	his.diva-portal.org Internet Source	<1%

10	journal.uad.ac.id Internet Source	<1 %
11	Konstantinos Koutsoumanis, Ana Allende, Avelino Álvarez‐Ordóñez, Declan Bolton et al. "Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain", EFSA Journal, 2021 Publication	<1 %
12	"Antibiotics and Antimicrobial Resistance Genes", Springer Science and Business Media LLC, 2020 Publication	<1 %
13	eurchembull.com Internet Source	<1 %
14	vital.seals.ac.za:8080 Internet Source	<1 %
15	STEPHANI LINGGAWAN. "Midline Granuloma", Hang Tuah Medical journal, 2017 Publication	<1 %
16	A.A. Dashti, R. Paton, S.G.B. Amyes. "Linkage of ciprofloxacin resistance with a single genotypic cluster of Klebsiella pneumoniae", International Journal of Antimicrobial Agents, 2006 Publication	<1 %

17	Paula Amador, Isabel M. Duarte, R.P. Roberto da Costa, Ruben Fernandes, Cristina Prudêncio. "Characterization of Antibiotic Resistance in Enterobacteriaceae From Agricultural Manure and Soil in Portugal", Soil Science, 2018 Publication	<1 %
18	cdnx.uobabylon.edu.iq Internet Source	<1 %
19	Jung-Im Yang. "A Survey of Staphylococcus aureus Contamination and Antibiotic Susceptibility in Retail Meat", Journal of the Korean Society of Food Science and Nutrition, 04/30/2008 Publication	<1 %
20	eprints.nottingham.ac.uk Internet Source	<1 %
21	J. Farber, K.-A. Moder, F. Layer, I. Tammer, W. Konig, B. Konig. "Extended-Spectrum Beta-Lactamase Detection with Different Panels for Automated Susceptibility Testing and with a Chromogenic Medium", Journal of Clinical Microbiology, 2008 Publication	<1 %
22	Naveed Ahmed, Kinza Tahir, Sara Aslam, Sara Masood Cheema et al. "Heavy Metal (Arsenic) Induced Antibiotic Resistance among	<1 %

Extended-Spectrum β-Lactamase (ESBL) Producing Bacteria of Nosocomial Origin", Pharmaceuticals, 2022
Publication

23	Tereza Gelbíčová, Kristýna Kořená, Lucie Pospíšilová-Hlucháňová, Nicol Straková, Renáta Karpíšková. "Dissemination and characteristics of Klebsiella spp. at the processed cheese plant", Czech Journal of Food Sciences, 2021 Publication	<1 %
24	downloads.hindawi.com Internet Source	<1 %
25	eprints.um.edu.my Internet Source	<1 %
26	"Antimicrobial Resistance in the Environment", Wiley, 2011 Publication	<1 %
27	Christiana Jesumirhewe, Adriana CABAL-ROSEL, Franz ALLERBERGER, Burkhard SPRINGER, Werner RUPPITSCH. "Genetic characterization of Escherichia coli and Klebsiella spp. from Humans and Poultry in Nigeria", Microbiology Society, 2023 Publication	<1 %
28	Golnaz Mobasseri, Cindy Shuan Ju Teh, Peck Toung Ooi, Kwai Lin Thong. "The emergence	<1 %

of colistin-resistant Klebsiella pneumoniae strains from swine in Malaysia", Journal of Global Antimicrobial Resistance, 2019
Publication

29	Qiu E. Yang, Uttapoln Tansawai, Diego O. Andrey, Shaolin Wang et al. "Environmental dissemination of mcr-1 positive Enterobacteriaceae by Chrysomya spp. (common blowfly): An increasing public health risk", Environment International, 2018 Publication	<1 %
30	Shaqiu Zhang, Muhammad Abbas, Mujeeb Ur Rehman, Yahui Huang et al. "Dissemination of antibiotic resistance genes (ARGs) via integrons in Escherichia coli: A risk to human health", Environmental Pollution, 2020 Publication	<1 %
31	research.bangor.ac.uk Internet Source	<1 %
32	uwspace.uwaterloo.ca Internet Source	<1 %
33	www.theses.fr Internet Source	<1 %
34	Anca Farkas, Brînduşa Bocoş, Anca Butiuc-Keul. "Antibiotic Resistance and intI1 Carriage in Waterborne Enterobacteriaceae", Water, Air, & Soil Pollution, 2016 Publication	<1 %

35	Kushneet Kaur Sodhi, Mohit Kumar, Dileep Kumar Singh. "Insight into the amoxicillin resistance, ecotoxicity, and remediation strategies", Journal of Water Process Engineering, 2021 Publication	<1 %
36	M. H. Effendi, I. G. Bintari, E. B. Aksono, I. P. Hermawan. "Detection of blaTEM Gene of Klebsiella pneumoniae Isolated from Swab of Food-Producing Animals in East Java", Tropical Animal Science Journal, 2018 Publication	<1 %
37	Noémi Nógrády, Judit Pászti, Henriett Pikó, Béla Nagy. " Class 1 integrons and their conjugal transfer with and without virulence-associated genes in extra-intestinal and intestinal of poultry ", Avian Pathology, 2007 Publication	<1 %
38	Yuvaneswary Veloo, Syahidiah S. A. Thahir, Sakshaleni Rajendiran, Lim K. Hock et al. "Multidrug-Resistant Gram-Negative Bacteria and Extended-Spectrum β-Lactamase-Producing Klebsiella pneumoniae from the Poultry Farm Environment", Microbiology Spectrum, 2022 Publication	<1 %
39	doi.org Internet Source	<1 %

40	dokumen.pub Internet Source	<1 %
41	e-sc.org Internet Source	<1 %
42	epubs.surrey.ac.uk Internet Source	<1 %
43	he02.tci-thaijo.org Internet Source	<1 %
44	laur.lau.edu.lb:8443 Internet Source	<1 %
45	mdpi.com Internet Source	<1 %
46	mspace.lib.umanitoba.ca Internet Source	<1 %
47	prr.hec.gov.pk Internet Source	<1 %
48	repositorio.utad.pt Internet Source	<1 %
49	static.frontiersin.org Internet Source	<1 %
50	www.ajol.info Internet Source	<1 %
51	www.ajvs.cl Internet Source	<1 %

52	www.researchgate.net Internet Source	<1%
53	Walid Elmonir, Norhan K. Abd El-Aziz, Yasmine H. Tartor, Samar M. Moustafa et al. "Emergence of Colistin and Carbapenem Resistance in Extended-Spectrum β-Lactamase Producing Klebsiella pneumoniae Isolated from Chickens and Humans in Egypt", Biology, 2021 Publication	<1%
54	"The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019", EFSA Journal, 2021 Publication	<1%
55	Juraj Majtan, Lubica Majtanova, Jana Bohova, Viktor Majtan. "Honeydew honey as a potent antibacterial agent in eradication of multi-drug resistant Stenotrophomonas maltophilia isolates from cancer patients", Phytotherapy Research, 2011 Publication	<1%
56	Reham A. Hosny, Mai A. Fadel. "Detection of Quorum Sensing N-Acyl-Homoserine Lactone Molecules Produced by Different Resistant Klebsiella pneumoniae Isolates Recovered from Poultry and Different Environmental	<1%

Niches", Applied Biochemistry and Biotechnology, 2021
Publication

57	W. P. Lokapirnasari, D. S. Nazar, T. Nurhajati, K. Supranianondo, A. B. Yulianto. "Production and assay of cellulolytic enzyme activity of Enterobacter cloacae WPL 214 isolated from bovine rumen fluid waste of Surabaya abbatoir, Indonesia", Veterinary World, 2015 Publication	<1 %

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

CASES OF MULTI DRUG RESISTANCE (MDR) IN KLEBSIELLA PNEUMONIAE ISOLATED FROM HEALTHY PIGS

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7