

# A Review of the Opportunistic Pathogen *Citrobacter Freundii* in Piglets Post Weaning : Public Health Importance

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## ABSTRACT

The review study will describe clearly related research about *Citrobacter freundii* in piglet intestines which has been known to play a very important role in animal health and its effects on aspects of public health. Thus, the existence of *Citrobacter freundii* as a pathogenic opportunistic bacterium as a result of weaning plays an important role in the formation and maintenance of the intestinal microbiota which affects the health and growth performance of pigs in the future. The review discussed the presence of *Citrobacter freundii* in the intestines of healthy pigs and its presence during the weaning period. The review explains comprehensively related research findings regarding the cytotoxicity of *Citrobacter freundii* and its resistance to several antimicrobials and provide an overview of recent studies that could help facilitate the design of a new strategy for conducting further research on *Citrobacter freundii* in pigs.

**Keywords:** *Citrobacter freundii*, microbiota, piglets, antimicrobial resistance, public health

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## INTRODUCTION

Scraps are home to diverse communities of microbial species that have a symbiotic link to pigs. In maintaining the dietary, physiological, and immunological functions of pigs, the gut microbiot population, or microbiota, has an important role[1]. But the microbial community also comprises species that cause disease such as *Escherichia coli*, *Salmonella*, *Clostridia* and *Citrobacter*. The disease and death of these pathogenic organs will occur. Thus, in the livestock industry the use of prophylactic antibiotics was widely used. Disturbance during pig breeding of the intestinal microbial ecosystem will significantly increase disease risk. Piglets are weaned until a healthy microbial population is formed and the immune system matures in order to speed up their development. Weaning stress will interact with the intestinal microbial ecosystem[2], thus rendering it more vulnerable to post-weaning bacteria [3]. This condition is prevented with prophylactic antibiotics, which decreases the occurrence of GDD, particularly when weaning. Increased antimicrobial resistance has been caused by prophylactic antibiotics and by growth-foster antibiotics and is now a significant global public health problem[4].

*Citrobacter Freundii* is a *Citrobacter* family bacteria which is a natural environment in both the human and animal intestinal tract[5]. *Citrobacter Freundii* is a member of the genus *Citrobacter*. But that is *C. Freundii* can also contribute to diarrhoea and other human infections[6]. A few *C. Freundii* isolates are characterised by virulence and cause human food poisoning or diarrhea[5]. The major factors of virulence observed in *C.*

caused diarrhoea. *Freundii* are toxins that are homologated to cholera subunitB[5], including toxins such as shiga, a heat-stable toxin.

In order to shape the microbial communities of the pig's intestines, the microbial composition of the intestines is established by various internal and external influences such as diet changes as well as probiotic and prebiotic provision and antibiotic supplementation in the feed [7]. Weaning is therefore an essential period of development and stress in animals in the early stages of life. The intestinal dynamics of piglets, especially *Citrobacter* bacteria during the weaning transition, are therefore interesting because they affect the health and production of growth of piglets. The results of the most recent and relevant research, which focuses on *Citrobacter* in the intestines of piglets, that affect piglets health, will be examined in this review.

## **Citrobacter the member of Enterobacteriaceae**

Both *Enterobacteriaceae* members share such bacteriological characteristics. It belongs to the gram-negative, rod-shaped and optional anaerobic community. Species of 1.0 x 2.0-6.0 µm *Citro* bacteria. The bacteria are solitary or colonial, have no capsule and are motile. *C.* In the medium of MacConkey Agar, the *freundii* shaped small, round, dark pink convex colonies. Also recorded were rough or slimy forms. At a certain temperature of 37 °C, *Citrobacter* grows optimally[8]. *Citrobacter* is present in a range of environmental sources, including soil and water, and in human and animal digestive tracts. These bacteria rarely cause illness, although some species can lead to infection of the urinary tract, sepsis and

meningitis in children. These bacteria play an important part in public health as pathogenic opportunisms in the incidence of food-borne illness.

A major taxonomic revision has taken place in the *Citrobacter* genus, using a new DNA-based technology. This genus is made up of 11 species called: *Freundii* *Citrobacter*, *C. (C. diversus)*, formerly), *C. C. Amalonacea*, *C. Oh*, youth, *c. Farmhouse*, *C. Farmhouse. braakii*, *c. braakii* *Werkmani*, *C. Werkmanii* *Sedlakii*, *C. Sedlakii*. *C. murliniae* and *C. Gillenii* *Rhodes* [9]. Everything except *C. Everything* animals. *Rodentium* (mice pathogen) from human clinical specimens have been described. But that is *C. C* and *freundii*. *C* and *Koseri*. The most significant pathogens of human beings are *braakii*. In water, the soil, the food and intestinal tract of animals and humans, *Citrobacter braakii* is commonly found [10]. *C. In* the peritoneum of the patient, *braakii* can cause acute peritonitis [11]. Eight isolates of *C* were identified in a study carried out by Liu *et al.* [6]. Two of them came out from pork. *Braakii* was from food. Additional studies to decide if *C* is needed. *Braakii* is a diarrheal disease contributor.

#### Composition of *Citrobacter* in healthy pigs

As an Enterobacteriaceae member of the intestine of each individual pig, the population of *Citrobacter* varies widely [12]. If Enterobacteriaceae is excluded, *E. The* bulk of the sample was still dominated by *coli*. *E* is the most common component of the pig intestinal Enterobacteriaceae. *Craftsman*. However, the amount of *E* in such samples. Often *coli* was lower than Enterobacterium cloacae and *Citrobacter Freundii* and *Klebsiella pneumoniae*. Dominant species or *E* species. *Coli* that prevails in certain regions of the intestine, other sections of the intestine are not generally identified. Various *E* colonies. In the intestinal tract of pigs, *coli* are always present whereas other Enterobacteriaceae are often present in a particular intestinal colony [13]. This indicates that only one colony of species other than *E* can be included in the observations. *Coli* in some part of the gut or just one colony dominating at all times, so it can't be found in other colonies. However, since Enterobacteriaceae species other than *E*, another possibility may occur. In animal populations, *coli* may also be very diverse. In a previous study in Australia there was high diversity with *C* of the gut Enterobacteriaceae of many mammals. *Fridays* [14]. *Freunday*. *C. Friday* *E. E. Coli*, *E. Coli*, *E. And* *K. Cloacae*. As dominant Enterobacteriaceae in pigs, *pneumoniae* were all isolated. Distribution information for hosts other than *E* from Enterobacteriaceae. The pathogenicity of *coli* is normally related. *C. Friday*, *E. Smith*, *K. Apples* and *L. Apples*. Skilled pathogens are known as *adecarboxylates*. Most data related to this species relate to human infection [15].

Pigs have also been shown to have *citrobacter* gallbladder populations [16]. 93 samples had bacteria out of the 145 gallbladder samples investigated and 79 were Gram-negative bacteria. Three were *Citrobacter freundii*, fifteen of them *Salmonella* spp.; forty-three were *Escherichia coli*, and three were of *Klebsiella* spp.; three were of *Aeromonas hydrophila*; three were of *Kronobacter sakazakii* and *E. T* and *fergusoni*. One *Guamensis*, one *Enterobacter* spp, and one 7 each. While its clinical value is not well known to animals, it can cause diarrhoea to resemble mild salmonellosis, much as in humans. In addition, carriers may be a cause of human enteric problems. *C. Freundii* is a commensal microorganism which isolated from severe infections in humans [17] but

which has not been widely reported to be of clinical significance to animals. Its pathogenicity is due to the resistance 's multi-drug nature [18]. It can affect the course of an infectious disease and prevent immune response and the host-pathogen interaction [19]. its capacity to gain resilience genes is important. Pathogenic microorganisms can therefore live in the ball blower of pigs temporarily or indefinitely, thereby becoming a reservoir for Gram negative bacteria contaminating pig products and infecting consumers.

#### Weaning transition and effect of *Citrobacter freundii* on the intestinal microbiota of piglets

Weaning is the most important thing in increasing production to improve the productivity of pig production .. The weaning is the sudden separation of the piglets from the sow which leads to stressful changes which can cause intestinal and immune problems, which can lead to lower health and growth output of the pigs [20]. Piglets are usually subjected to a number of stressors during the weaning period, including the abrupt shift in the diet from milk to solid vegetable food. The degradation induces physiological changes in the intestinal structure and function [21]. In addition, after inducing different causes, the intestinal microbiota of piglets is induced during weaning rather rapidly. During the pig's transition time the change in commensal bacterial structure is referred to as a microbial shift [22].

One of the key factors that influence the transition from simple to more complex nutrient sources in microbiota piglets during weaning is the sudden shift in diets which can influence the absorption ability of the small intestine and probably affect growth and feed performance. The weaning period is of great importance because piglets are exposed to thousands of bacteria, that may play an important role in the construction of adult pigs' gut microbiota [23]. Microbial exposure is important in early life for rising, developing the immune system and pig health [24]. Furthermore, during the weaning period microbiota formation plays a major role because piglets are often immature and rely upon porkmilk to avoid colonisation and over-crop of opportunistic pathogens [25]. Therefore the gut microbial succession in the weaning transition has to be better understood and which factors affect swine gut microbial changes with a view to improving the digestive health, development, and well-being of piglets.

The weaning duration is an integral part of the pig's lives as the microbial composition of the intestines and the immune system continues to evolve, making swine vulnerable to pathogenic substances that cause post-weaning diarrhoea [26]. In the swine field, diarrhoea has become a major cause and increased death. In recent years, interesting evidence has been increased for the incidence of diarrhoea, not simply unique pathogenic agents, by the intestines microbiota [24]. The gut microbiota is recognised as defending against pathogens by controlled immune response from the host. The key group of bacteria of the terminal ileum of weaning piglets has been identified for example, segmented filamentous bacteria (SfB) or *Candidatus arthromitus*, recognised as modulators of the Immune system mammalian [27].

Piglets undergo a temporary decline in feed or anorexia during the weaning process which can lead to inflammation of the intestines [28]. In a recent study, colitis leading to a microbiota imbalance [30] has been the mechanism of Guevarra *et al.* The host reaction in intestinal conditions induces reactive oxygen, since nitric

(NO) oxide can be rapidly transformed from released into the intestinal lumen into nitrate (NO<sub>3</sub><sup>-</sup>). [30] The rich nitrate environment is very beneficial to Enterobacteriaceae growth that covers the gene nitrate reductase [31]. Several enterotoxigenic E pathogens in the family of enterobaceae, namely Salmonella enterica serovar Typhimurium. Coli (ETEC), caused by pigs, can cause intestinal inflammation, disrupting the composition of microbiota [32, 33]. In studying the Salmonella typhimurium model, Arguello *et al.* [34] showed that there was a decline in the populations of bacteria needed, such as bifidobacterium, and lactobacillus, with an increase in pathogenic bacteria as Citrobacter and anaerobic decrease, namely Clostridium, Ruminococcus. Or diallister on weaned piglets' ileal mucosa. Intestinal inflammation due to weaning thus disrupts the intestinal microbiota, promoting in particular Enterobacteriaceae, which promotes the growth of enteropathogenic bacterias. More study is therefore required to understand the effect of colitis on intestinal microbiota impairments in piglets after weaning [35].

#### **Cytotoxicity of *Citrobacter freundii* in Pigs**

A study conducted by Bai *et al.*, [5] in a village in China, has found 26 Citrobacter isolates into 20 pulse types, and 2 of them from pigs and have cytotoxic activity. The isolates obtained from humans and animals have the same types of pulse. It was also found that one animal and fly isolate had the same type of pulse. This suggests that animals can act as reservoirs for human infection and flies may act as carriers of transmission. Flies are known to transmit bacteria between humans. In a study flies reported that not only as a mechanical vector, but bacteria have been found to multiply in the mouth of flies and excreted through fly feces. Both isolates have cytotoxicity and adhesion activities to HEp-2 cells. Determination of the cytotoxicity of bacteria to cells is carried out by measuring the lactate dehydrogenase produced by the cells. This treatment causes the cells to release 24.3% dehydrogenated lactate (LDH) which causes damage and cell death of > 50%.

Another study conducted by Tschape *et al.*, [15] found the verotoxinogenic properties of Citrobacter freundii which contaminated vegetables used in sandwiches in a nursing school. Vegetables contaminated with verotoxin from Citrobacter freundii are caused by the use of manure from pig feces in organic gardens. This incident causes severe gastroenteritis and haemolytic uraemic syndrome. Of the 152 people, 36 had positive test results for verotoxin using the ELISA testing method. And surprisingly, 59 isolates were found including C. freundii from 72 isolates and the rest were 13 isolates from E. Coli. This examination was performed using the dot blot hybridization techniques method on positive stool samples.

Research conducted by Liu *et al.*, [6] in the Maanshan Ahui Province of China isolated Citrobacter spp. from patients with diarrhea, foodstuffs, and some of them come from pork and pork. Of the 62 isolates tested, four isolates (including three C. youngae and one C. freundii) showed the strongest adhesion, with an adhesion index of > 50.25 isolates showed moderate adhesion, with an adhesion index between 1 to 50. Nineteen isolates showed weak adhesion, with adhesion index <1. The remaining isolates showed ambivalent or no adhesion. The lowest adhesion rates of each type of bacteria were C. braakii (25%), C. freundii (77%) and C. youngae (88%). There was no significant difference (P > 0.05) of any

adhesion between several sources (human and food) of Citrobacter isolates.

Another study conducted by Tajeddin *et al.*, [37] regarding the outbreak in the Islamic Republic of Iran, found 19 isolates of Citrobacter out of 484 samples tested. Of the 19 Citrobacter isolates obtained, two of them carried the stx1 gene. In his research, this was the first time an outbreak was caused by foodborne illness with stx1 encoded by the C. freundii strain. Since only a small proportion of these strains carry the stx1 gene, it is possible that other virulence factors exist in these bacteria. Other virulence factors that have been reported for diarrhea associated with C. freundii include a heat-stable toxin, cholera-like eae and aea. The presence of stx1 encoded by the C. freundii strain in diarrhea in this study is significant, although the clinical impact and role of this emerging strain in human pathogenicity have not been reported. The spread of Shiga toxin-producing phages by horizontal gene transfer can occur via environmental stimulation, as may antibiotics be the cause of this emergence [38].

Another study conducted by Schmidt *et al.*, [39] showed that Citrobacter freundii can produce Shiga-like Toxin II-Related Cytotoxin isolated from patients with diarrhea and meat. In this incident, to determine the presence of slt-gens associated with Citrobacter freundii, an examination was carried out by colony hybridization, PCR analysis, and nucleotide sequence. In testing based on the toxicity of Citrobacter freundii it can produce functional toxins. In his research, he found a similarity with the genes produced by the Escherichia coli slt-II group. It is suspected that this happened because of the transfer of genes between the two bacteria. Because when the neutralization test was carried out, this study showed that the cytotoxic activity of the C. freundii isolates could be neutralized by antibodies against SLT-II produced by Escherichia coli. To ensure the existence of a relationship between the generator between the two bacteria was examined using PCR and found that C. freundii had different results and determined that C. freundii had slt-licA and slt-IIcB genes toxins. This is because Shiga toxin-encoding phages are highly mobile both in regulation and transverse horizontally due to environmental influences such as exposure to antibiotics [38]. In general, lysogenic phages are important particles in population dynamics within the host, which are useful for killing sensitive bacteria and converting lysogenic to viable bacteria [40]. Guarino *et al.*, [41] have also successfully detected the presence of 18-amino-acid heat-stable enterotoxin produced by Citrobacter freundii and which is similar to 18-amino-acid Escherichia coli heat-stable enterotoxin. The role of the toxin produced by Citrobacter sp. in inducing diarrhea is not known, but the toxin from Citrobacter sp. has a biological potency similar to the toxin from Escherichia coli. Furthermore, this study indicated the presence of a strain of Citrobacter sp. which can produce 75% more toxins than Escherichia coli, which isolates Citrobacter sp. This optimization was carried out under the same conditions as E. coli.

#### **Antimicrobial Resistance Profile of *Citrobacter freundii* in Pig**

C. Freundii is a commensal, but unstudied micro-organism, which is mostly isolated from severe nosocomial infections in human beings [31] because it is clinically significant in animals. Its disorder is related to a multi-drug resistance [18]. In a study by Evangelopoulou *et al.*, the resistance level of C was tested. Pig galbalader

isolated *freundii* showed resistance to 20 of the 24 antibiotics examined. Antibiotics are amoxicillin (30 µg), aztreonam (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), cefazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), colistin (50 µg), enrofloxacin (5 µg), gentamicin, gentamicin (10 µg) and enrofloxacin (15 µg), amoxicillin (30 µg), aztreonam (30 µg), cefoxitin (30 µg), ceftiofur (30 µg), Cef of the antibiotics C of all these. Aztreonam, cefotaxime, tigecycline, and doripenem were not immune to *freundii*. The test uses the process of disc diffusion [43]. The developmental capacity to acquire resistance genes affects the trajectory of an infection, immune-response prevention and interactions with host pathogens [19]. The key reservoir of multidrug resistance for multidrug-resistant bacteria showed that pigs are the most significant. The use of antimicrobials in the feed helps to excrete gram-negative enteric pathogens which cause pork to contaminate pork products that can lead to consumer infection [44].

Multidrug resistance can be caused by the transfer of resistance genes from the various resistor genes encoded in a resistant microbe to different bacterial species encoding related antimicrobial classes [45]. Resistance to antimicrobials can be transmitted to both pathogenic and commensal pathogens [46,47], such as gram-negative bacteria [48-55] and gram-positive pathogens [56-62]. Antimicrobial resistance can also be transmitted by animal products as a virulence factor [63-67]. This can have major effects on the growth of bacteria in natural bacterial populations and may contribute to infectious diseases [19] as a way to escape the immune system. Pathogenic microorganisms may also live indefinitely or temporarily in a pig's bile blood and become a multidrug reservoir of Gram's negative bacteria, which may contaminate pork products and infect consumers.

Liu et al. also tested 82 C isolates [6] in an alternative analysis. *Freundii* against 17 antibiotics belonging to 10 classes of antibiotics using the CLSI recommended disc diffusion process. The 82 isolates of mainly C. *Freundii* have been immune to β-lactams, in particular penicillin (41.5%), cephalosporins (19.5%–98.8%) and monobactam (25%). Resistance to two quinolones: ciprofloxacin (7.3%) and levofloxacin (2.4%), aminoglycosides (2.4%–11.0%); phenicol (2.4%), sulfonamides (6.1%), tetracycline (8.5%), and nitrofurantoin (13.4%) respectively. The prevalence of resistance to cephalosporins of first generation, such as cefazolin, was 98.8% and cefoxitin of second generation was 74.4%. In general, ceftriaxone (28.0%), ceftazidim (29.3%) and cefepime (19.5%) were less resistant in this sample; in the present study, 26 isolates were multi drug (MDR), with an antibiotic resistance of three or more different class (MDR) groups.

Another study by Liu et al. [68] which isolates *Citrobacter*, found 21 isolates of *Citrobacter* after E from pork samples, pig faeces and pork farms. Craftsman. 13 isolates were multidrug resistant out of 21 isolates obtained from *Citrobacter*. High frequencies of gentamicin (98% of the isolates), streptomycin (98%), tetracycline (98%), chloramphenicol (98%), kanamycin (96%), doxycycline (80%), ciprofloxacin have been presented. (74.1%), nalidixic acid (97.4%), ampicillin (64.7%), and amoxicillin-clavulanic (60.8%) percent. (94.0%), nalidixic acid (94.1%) and trimethoprim (94.1%). In general, sulfamethoxazole, trimethoprim, tetracycline, gentamicin, streptomycin and chloramphenicol resistant isolates. In his study, 50% of the *Citrobacter* isolates isolated from

pork, but some have different antibiotic resistance profiles and are characterised by different resistant genes, to 100% homology with environmental isolates. Several recent studies have found that *Citrobacter* isolated from pork samples and hog farm conditions have antibiotic resistance [69,70]. *Citrobacter* Research has shown that pork and pig farms can be sources of resistant bacteria which can be passed on to humans by meat products in industrial chains.

## CONCLUSION

In this review it can be concluded that the microbial ecosystem in the digestive tract of piglets plays an important role in physiological functions and antibody formation. The presence of digestive tract disorders caused by factors such as stress as a result of weaning can disrupt the microbiota system in the digestive tract of piglets. This can provide opportunities for pathogenic opportunistic bacteria such as *Citrobacter freundii* to grow rapidly to form colonies that can cause disease. *Citrobacter* has the ability to produce toxins that are resistant to heating and produce shiga-like toxins which of course can disrupt the balance of the microbiota composition in the intestines of pigs. In addition, these bacteria also have the ability to mutually transfer toxin coding between other bacterial groups such as *E. coli*. Many recent studies also reveal that in general *Citrobacter freundii* is MDR which causes these bacteria to be difficult to control with certain antibiotics. In addition, the threat of *Citrobacter freundii* which is MDR can also act as a foodborne disease in meat that can potentially be transmitted to humans and have an impact on public health. In addition, these bacteria can also pollute the environment such as soil and water. The importance of further understanding about the threat of *Citrobacter freundii* which is MDR requires special attention to determine the policy of using antibiotics in a pig farm.

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