Antimicrobial-resistant *Klebsiella* species isolated from free-range chicken samples in an informal settlement

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Abstract

Introduction: Sub-therapeutic doses of antimicrobial agents are administered routinely to poultry to aid growth and to prevent disease, with prolonged exposure often resulting in bacterial resistance. Crossover of antibiotic resistant bacteria from poultry to humans poses a risk to human health.
Material and methods: In this study, 17 chicken samples collected from a vendor operating in an informal settlement in the Cape Town Metropolitan area, South Africa were screened for antimicrobial-resistant Gram-negative bacilli using the Kirby Bauer disk diffusion assay.
Results: In total, six antibiotics were screened: ampicillin, ciprofloxacin, gentamicin, nalidixic acid, tetracycline and trimethoprim. Surprisingly, *Klebsiella ozaenae* was identified in 96 and *K. rhinoscleromatis* in 6 (n = 102) of the samples tested. Interestingly, ~40% of the isolated *Klebsiella* spp. showed multiple resistance to at least three of the six antibiotics tested.
Conclusions: *Klebsiella ozaenae* and *K. rhinoscleromatis* cause clinical chronic rhinitis and are almost exclusively associated with people living in areas of poor hygiene.

Key words: antibiotic-resistant, poultry, resistance, *Klebsiella* species.

Introduction

Antimicrobial agents are frequently used in food animals for the promotion of growth [1]. The latter is achieved by the antibiotics decreasing the susceptibility of the animals to bacterial infections and by increasing food absorption in the intestine. Of great concern is the fact that these antimicrobial agents are the same, or closely related, to antimicrobials used in human medicine [1]. Also, as a result of the widespread use of these antimicrobial agents, bacteria have developed ways to circumvent the effects of antibiotics through evolutionary adaptations [2].

Therefore, contamination of food with antibiotic-resistant bacteria poses a major threat to public health and the transfer of these antibiotic-resistant traits to pathogenic bacteria could potentially compromise the treatment of bacterial infections in the clinical setting [3].

Members of the genus *Klebsiella*, especially *K. pneumonia* and *K. oxytoca*, are opportunistic pathogens associated with severe nosocomial infec-

tions such as septicaemia, pneumonia and urinary tract infections. *K. pneumonia* has been taxonomically subdivided into three subspecies: *K. pneumonia* subsp. *pneumonia*, *K. pneumonia* subsp. *ozaenae* and *K. pneumonia* subsp. *rhinoscleromatis* [4]. *Klebsiella rhinoscleromatis* and *K. ozaenae* cause two clinical forms of chronic rhinitis, namely rhinoscleroma and ozena, respectively [5]. Both diseases are endemic in areas with poor hygiene conditions and are commonly not detected in developed countries [6, 7]. Even though the isolation of antimicrobial-resistant and -susceptible strains of *Klebsiella* from poultry have previously been reported [8, 9], this is, to our knowledge, the first report of the isolation of multiple-antibiotic-resistant *Klebsiella rhinoscleromatis* and *K. ozaenae* isolated from chicken samples.

Material and methods

Sample collection

Free-range chicken samples (n = 17) were collected from street vendors from the informal settlement of Langa, in the Western Cape Province of South Africa. Neck-skin samples were collected aseptically in sterile sample bags, refrigerated upon arrival in the laboratory and processed for microbiological examination within 24 h.

Microbiological analysis

Twenty-five grams of neck skin was aseptically weighed and placed into a sterile polyethylene stomacher bag containing 225 ml of Buffered Peptone Water (BPW) (Oxoid, CM0509). Each sample was homogenized for 120 s at normal speed using a Seward stomacher. One ml of the homogenate was then transferred into 9 ml sterile BPW and 10-fold serial dilutions were performed (10^{-1}-10^{-6}). A 100 µl aliquot of each dilution was then spread-plated onto plate count agar (PCA) and incubated for 18 h at 37°C. Following overnight incubation, bacterial colonies were enumerated to determine the total aerobic bacteria count.

Additionally, after 6 h of incubation at 37°C, 100 µl of the pre-enriched sample was pipetted from each dilution series into 9.9 ml Rappaport-Vassiliadis soya peptone broth (RV) (Oxoid, CM0669) and incubated overnight at 37°C. After 24 h of incubation, growth in tubes with the highest dilution were selected from each sample and streaked onto three Xylose Lysine Deoxycholate (XLD) (Oxoid, CM0469) plates and three Brilliant Green Agar (BGA) (Oxoid, CM0263) plates. These respective selective media allow for the presumptive identification of *Enterobacteriaceae*; XLD also differentiates between pathogenic *Enterobacteriaceae*. The agar plates were then incubated overnight at 37°C.

Identification of *Klebsiella* spp.

After the overnight incubation, one isolate from each of the three XLD and BGA plates was selected and emulsified in 5 ml of sterile saline solution. The isolates were identified using the MicrobactTM 12B identification kit according to the manufacturer's instructions (Oxoid).

Antibiotic susceptibility tests

Isolates were screened for antibiotic resistance using the Kirby-Bauer disk diffusion assay. For each sample, three colonies were selected from both XLD and BGA plates respectively, and inoculated into Tryptone Soya Broth (TSB) (Oxoid, CM129). Thus, six isolates were obtained from each sample to ensure reproducibility of the results. The cultures were incubated for 4 h at 37°C with continuous shaking. A 100 µl of inoculum was then spread onto Mueller-Hinton Agar (MHA) (Oxoid, CM0337). Antibiotic disks were dispensed onto the plates and incubated overnight at 37°C. Isolates were tested for susceptibility to six different antimicrobial agents, namely ampicillin (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg) and trimethoprim (1.25 µg). The CLSI [10] guidelines were utilized to classify the isolates as (i) resistant, (ii) intermediate-resistant or (iii) susceptible to the various antibiotics. Isolates exhibiting intermediate resistance were classified as resistant.

Multiple antibiotic resistance

The antibiotic code profile developed by Manie *et al*. [11] was used to determine whether the isolates were displaying multiple antibiotic resistance (MAR) (see supplemental data for explanation).

Results

In this study, of the 102 *Enterobacteriaceae* isolated using conventional selective media, 96 were identified as *Klebsiella ozaenae* and 6 as *K. rhinoscleromatis* with the Microbact 12B identification kit. The resistance of these *Klebsiella* spp. to six antibiotics was determined using the Kirby-Bauer disk diffusion assay. Isolates showed high levels of resistance; 66.7% were resistant to ampicillin, 61.8% to nalidixic acid, 59.8% to tetracycline and 50% to trimethoprim (Table I). In fact, of greater concern, the antibiotic code profiles showed that ~40% of the isolated strains showed multiple antibiotic resistance (MAR) to three or more antibiotics: (i) 21.5% of the *Klebsiella* spp. showed resistance to ampicillin, trimethoprim, nalidixic acid and tetracycline, (ii) 9.8% showed resistance to ampicillin, nalidixic acid and tetracycline and (iii) 8.8% showed resistance to ampicillin, trimethoprim and nalidixic acid (Table II, see Appendix).

Table I. Antimicrobial resistance patterns of *Klebsiella* spp. isolates from informal settlement

Antibiotic used	% of resistant isolates (n = 102)
Ampicilin	66.7 (68)
Ciprofloxacin	4.8 (5)
Gentamicin	3.9 (4)
Naladixic acid	61.8 (63)
Tetracycline	59.8 (72)
Trimethoprim	50.0 (60)

Table II. The multiple antibiotic resistance code profile of *Klebsiella* spp. isolated from free-range chickens

MAR code	% isolates resistant
01	2.95
02	0.98
0.4	10.78
0.6	1.96
10	10.78
11	3.92
12	3.92
13	0.98
14	3.92
16	9.80
36	0.98
42	5.89
46	8.82
50	0.98
52	5.89
53	0.98
54	0.98
56	21.56
57	0.98
76	2.95

Discussion

Antibiotics, such as bacitracin, chlortetracycline, erythromycin and penicillin, are routinely used for control and treatment of bacterial diseases in poultry. When these antibiotics are administered to the birds over extended periods, especially at low levels, certain species of bacteria become resistant [9]. Alarmingly, more and more scientific evidence shows that these resistant bacteria, including pathogens, can be transferred to humans through the food chain [12].

In the clinical setting, an adequate and prolonged antimicrobial regimen is needed to treat the two types of chronic rhinitis caused by *K. ozaenae* and *K. rhinoscleromatis* [13]. *Klebsiella rhinoscleromatis* is normally sensitive to the majority of antibiotics that are active on Gram-negative bacteria except for penicillin, ampicillin and sulfonamide [14], with ciprofloxacin and rifampin the most effective antimicrobials [15]; in terms of efficacy/cost, the association of trimethoprim-sulfamethoxazole is the best compromise [7]. Botelho-Nevers *et al*. also reported good results when using rifampin associated with co-trimoxazole to treat *K. rhinoscleromatis*. Limited antimicrobial susceptibility studies of *K. ozaenae* have shown varying degrees of susceptibility to ampicillin [13, 16, 17].

There is evidence that the routine use of antibiotics in animal husbandry leads to antibiotic resistance in bacteria. These antibiotic-resistant bacteria can infect or reach the human population not only by direct contact, but also by food products of animal origin [18]. Therefore, the reduction and eventual elimination of antibiotics for purposes other than veterinary therapy or treatment of infections in animals is essential. This can be achieved by improving methods of animal husbandry, the eradication of diseases in animals, the optimal use of existing vaccines and the development of new vaccines [18, 19]. Generally, these interventions are aimed at reducing the development and incidence of resistant bacterial infections, thereby prolonging or restoring the effectiveness of existing antibiotics [20]. Importantly, a good programme for prevention of antibiotic resistance also includes an active system of surveillance for resistance, an active and effective infection control programme to minimize secondary spread of resistance, and the sensible use of antimicrobials in animal production systems [21].

This manuscript reported the isolation of multiple-antibiotic-resistant *K. rhinoscleromatis* and *K. ozaenae* from chicken samples collected from a vendor in an informal settlement in South Africa. Surprisingly, resistance to antibiotics commonly used in the clinical treatment of *K. rhinoscleromatis* and *K. ozaenae* infection was observed. This could pose a serious health risk if vertical transmission occurs between infected poultry samples and humans.

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Appendix

Multiple antibiotic resistance. Briefly, the antibiotics were divided into two groups and each antibiotic was designated a particular number. Group 1, which contained ampicillin, was designated number 1, ciprofloxacin was designated number 2 and trimethoprim was designated number 4; group 2 contained gentamicin and was also designated number 1, nalidixic acid was designated number 2 and tetracycline was designated number 4. If an isolate was resistant to a particular antibiotic it was given the number designated to that particular antibiotic. If the isolate was sensitive to the antibiotic it was given zero. The numbers awarded were added to yield the respective code. For example, an isolate resistant to ampicillin, ciprofloxacin and gentamicin, but sensitive to the other antibiotics would receive the code (1 + 2 + 0) (1 + 0 + 0) to give a profile of 31.