Drug Resistance in Enteroptahogens of Zoonotic Significance Isolated from Diarrhoeic Pigs and House Sparrow Perching in Piggery in Jharnapani, Nagaland

Bhoj Raj Singh

Indian Veterinary Research Institute Izatnagar Bareilly Uttar Pradesh INDIA

Abstract
In the study on 17 pooled fecal dropping samples of house sparrow (Passer domesticus) perching in a piggery and 38 rectal swabs from diarrhoeic pigs in the piggery for aerobically growing enteric bacteria a total of 34 enterococci and 39 Gram negative (G-ve) bacteria were isolated from droppings of the birds. From diarrhoeic pigs, 40 Gram positive and 54 G-ve bacteria were isolated. About 52.9% G+ve and 38.5% G-ve bacterial isolates from house sparrow were resistant to three or more drugs (MDR) and belonged to 18 and 9 MDR patterns, respectively. Twenty four G+ve and 29 G-ve MDR strains from pigs could be classified in to 16 and 22 MDR patterns, respectively. Circulation of common bacteria could not be identified among pigs and house sparrow as none of the bacterial strains belonging to the same species isolated from house sparrow as well as from pig samples had similar antibiotic sensitivity pattern.

Keywords: Enterococcus, Streptococcus, E. coli, Edwardsiella tarda, Salmonella, Aeromonas caviae

Enteropathogens in pigs and and house sparrow perching in the piggery differs significantly however the drug resistance pattern was often similar. Bacteria may not be as fast and frequent in spreading from one host to other as the drug resistant genes.

1. Introduction
Enteric bacteria are very important for human and animal health because they being opportunistic pathogens may cause disease in human, animals and birds and spread through faeco-oral route in many forms and persist in environment for long (1, 2). Besides being known reservoir for several enteropathogens including Campylobacter, Salmonella, toxigenic E. coli and many other opportunistic pathogens, birds can spread more than 60 diseases either through their droppings or through direct contacts (3-5). Birds have been responsible for several disease outbreaks in human, animals and domesticated birds due to their extensive mobility (6, 7). Studies indicated that antimicrobial resistance (AMR, specially due to production of extended spectrum -lactamases) among bacteria isolated from free living birds is common, however those birds which have never come in contact of human or domesticated animals AMR is of occasional occurrence (8). Birds have been identified as the melting pot for emergence of new drug resistant types of strains and also the reservoir for the new types of drug resistant strains; therefore, mapping of drug resistance of the bacterial strains from birds may be one of the best indicators for monitoring the emergence of drug resistance in bacteria (9). Birds acquire enteropathogens through feeding on raw sewage, dead animals and garbage, and then spread these pathogens to humans directly or by contaminating commercial/ backyard poultry units in a wide area of their activity even globally, analogous to air-travelers (4, 5, 10-12). Similarly in pigs, around 500 different species of microorganisms have been described
and identified to inhabit pig intestine (13, 14) belonging mostly to Streptococcus, Lactobacillus, Bifidobacterium, Enterococcus, Eubacterium, Fusobacterium, Peptostreptococcus, Enterobacter, Bacteroides, Porphyromona, Escherichia and Clostridium spp. (14-17). Although avirulent strains of E. coli are the commonest constituent of intestinal microflora, pathogenic strains of the same bacteria are the most frequent causes of post weaning diarrhoea (PWD) in piglets (18, 19). Many of the enteric bacteria of pigs are also common in other animals and birds. There are reports of dozens of bacteria associated with diarrhea in pigs; however, E. coli dominates the scene followed by Clostridium spp., Campylobacter spp., salmonellae, enterococci, Yersinia enterocolitica and Streptococcus suis (20). Besides, Actinobacillus infections, Bacteroides infection, grey scour syndrome, leptospirosis and Pseudomonas infections are also reported as cause in many cases of pig diarrhoea (21). This study, enteric bacteria isolated from faecal droppings of house sparrow (Passer domesticus) perching in piggery, were compared with enteric bacteria isolated from rectal swabs of diarrhoeic pigs for a better understanding of circulation of enteropathogens and their drug resistance pattern.

2. Materials and Methods

Samples: A total of 38 rectal swabs were collected over a period of one year (April 2009 to March 2010) from pigs suffering from diarrhoea in an organized piggery having large black and local Nagaland pigs at Jharnapani, Dimapur, Nagaland. On all the occasions of diarrhoeic diseases in pigs, two to three house sparrow (Passer domesticus) perching sites in the piggery were identified and apparently fresh faecal droppings were collected. Dropping collected at a particular time and from a specified site were pooled to make one sample. A total of 17 samples were collected on the occasions when diarrhea was observed in three or more pigs. Pooled faecal dropping samples and rectal swabs were brought to laboratory within 30 min of collection in sterile cotton plugged tubes. Isolation and identification of bacteria: Rectal swabs and birds dropping were transferred to 10 and 50 ml of buffered peptone water (BPW, Hi-Media, Mumbai), respectively, shaken to suspend the solid if any in the sample and incubated at 37°C for 6-8 h. From each tube, aliquots were streaked on to plates of Hektoen enteric agar (HEA, Hi-Media), blood agar (Hi-Media) and Enterococcus selective agar (EA, Hi-Media). Plates were incubated at 37°C for 24-48 h. Three to five isolated colonies of each of different types were picked up from the petri-plates and characterized on the basis of morphology, culture characteristics and biochemical characteristics (22, 23).

Antibiotic sensitivity assay: All isolates except those of Enterococcus spp., and Streptococcus spp. were tested for their sensitivity pattern using disk diffusion assay on Mueller Hinton agar (MHA, Hi-Media) while enterococci and streptococci were tested similarly using brain heart infusion agar (BHIA, Hi-Media) plates (because of their poor growth on MHA) as per recommendations of CLSI (24). Bacterial isolates of the same species isolated from the same sample and having the same antimicrobial sensitivity pattern were considered as a single isolate while those differing in sensitivity were considered as different isolates. All Gram positive (G+ve) bacteria were tested against disks of 15 antimicrobials drugs (ampicillin, 30 g; amoxicillin+clavulanic acid, 10+5 g; chloramphenicol, 30 g; carbenicillin 30 g; clindamycin, 2 g; cephotaxime, 30 g; ciprofloxacin, 10 g; co-trimoxazole, 25 g; cephalxin, 30 g; cinoxacin 5 g; erythromycin, 15 g; gentamicin, 10 g; norfloxacin, 10 g; oxacillin, 5 g; tetracycline, 30 g) while Gram negative (G-ve) bacteria were tested against 11 antimicrobials including ampicillin (30 g), ceftazidime (30 g), cephalexin (10 g), cotrimoxazole (25 g), gentamicin (10 g), nalidixic acid (30 g), netilmicin (10 g), nitrofurantoin (300 g) and tetracycline (30 g). After 24 h of aerobic incubation at 37°C zone of inhibition was measured in millimeters (mm) and results were concluded as sensitive or resistant as per CLSI (24, 25). All the tests were repeated to conformity. Statistical analysis: Antibiotic sensitivity data entered in Microsoft Excel work sheet was analyzed to compare the results classified as sensitive or resistant with Chi-squared test (2). All test results were evaluated at 5% level of significance (p 0.05).

3. Results

Of the 17 samples of faecal droppings of house sparrow (Passer domesticus) perching in piggery, 34 enterococci and 39 G-ve bacteria were identified (Table 1). All samples contained two or more than two types of bacteria (Table 1). Of the 17 samples, one or more types of enterococci were identified from 15 samples. Enterococcus avium and E. gallinarum were isolated from 10 samples each while E. caecorum and E. faecalis were isolated from four and two samples, respectively. Among the G-ve isolates, Escherichia coli were the commonest and 14 strains were isolated from 11 samples. Other G-ve enteric bacteria iso-
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cluded included Klebsiella pneumoniae from four samples followed by Serratia ficaria (3 samples). Other bacteria isolated from one or two samples included E. fergusonii (1), E. blattae (1), Proteus mirabilis (2), Praga fontium (2), K. planticola (1), Serratia odorifera (1), S. ptyuthica (1), Citrobacter freundii (1), Enterobacter gregoviae (2), E. agglomerans (1) and Xenorhabdus bovienii (1). Among G+ve bacterial isolates from house sparrow 52.9% were resistant to three or more drugs but none of the two E. faecalis strain had multiple drug resistance (MDR) i.e, resistance to 3 antimicrobials of different classes. None of the 15 antimicrobials was either effective or ineffective on all of the enterococci. None of the enterococci from birds was sensitive to all the antimicrobials tested. A total of the 15 strains of enterococci were resistant to only one drug, one each to two, four, seven and 12 drugs, three each to three, five and nine drugs, two each to six, eight and 10 antimicrobials. Surprisingly more strains were resistant to carbenicillin (50%) than ampicillin (32.4%). Gentamicin (88.2%) and tetracycline (86.5%) were the most effective antimicrobials followed by cindamycin (79.4%), cipurofloxacin (79.4%), norfloxacin (76.5%), erythromycin (76.5%), chloramphenicol (73.5%), amoxicillin+clavulanic acid (73.5%), cephalexin (67.6%), cloxacillin (67.6%), cephalaxin (64.7%), oxacillin (58.8%) and co-trimoxazole (8.8%). Only 38.5% (15) of 39 G-ve strains isolated from house sparrow were resistant to three or more drugs. Of these, 13 were sensitive to all the 11 drugs tested. Four strains were resistant to one, seven strains to two, four strains to three, three strains each to four, five and six, and one strain each to 7 and 8 antimicrobials. From birds, none of the Enterobacter spp. (3), Serratia ficaria (4), S. ptyuthica (1), and X. bovienii (1) strains had MDR. Although 35.7% (5) strains of E. coli and 1 of the two strain of E. blattae were MDR type the only strain of E. fergusonii had no MDR. None of the 39 G-ve strains isolated from house sparrow was resistant to cephotaxime and gentamicin. Similar to the effect on enterococci, cotrimoxazole was the least effective drug on G-ve bacteria too, inhibiting only 56.4% strains. Resistance to netillan (41%) was surprisingly high and equal to ampicillin. Many of the strains were also resistant to ceftazidime (35.9%), tetracycline (30.8%), chloramphenicol (17.9%), nalidixic acid (10.3%), nitrofurantoin (10.3%) and ciprofloxacin (7.7%). G+ve and G-ve strains isolated from house sparrow could be classified in to 18 and 9 MDR patterns, respectively (Table 2). None of the two enterococci of different species had similar MDR patterns. Fifteen MDR strains G-ve bacteria fell in to nine patterns. Common antibiotic resistance patterns were evident in E. blattae, K. pneumoniae and K. planticola (A,Co,Nf); two strains of E. coli from different samples (Co,Na,T); K. pneumoniae and Praga fontium (A,Co,Na,Nf); E. coli, Proteus mirabilis and Praga fontium (A,Co,Na,Nf) strains. 2 statistics proved that there was no significant difference (p =0.05) among strains of different bacteria isolated from birds with respect to their resistance against most of the antimicrobials. However, resistance to nitrofurantoin was more common in K. pneumoniae strains (p=0.07). From 38 rectal swabs of diarrhoeic pigs a total of 40 G+ve and 54 G-ve bacteria were isolated (Table 3). Of the 38 samples, nine had only one type of bacteria. E. coli being the most commonly isolated one (from five samples), followed by Edwardsiella tarda (2), Enterococcus solitarius (1), and E. faecalis (1). Remaining 29 samples contained two (20), three (6), four (2) or five (1) types of enteric bacteria (Table 3). Escherichia coli were isolated from 26 samples followed by Streptococcus sanguis (16), Edwardsiella tarda (8), Enterococcus faecalis (5) and E. gallinarum (5). Some bacteria were isolated from one (K. pneumoniae, E. solitarius, E. faecium, Micrococcus agilis, Aeromonas caviae and Salmonella enterica subspecies enterica) or two (E. mundatii, E. gregoviae, E. caselliiflavus, E. dispar, C. freundii, Pseudomonas aeruginosa and Proteus vulgaris) rectal swabs only. Twenty four G+ve and 29 G-ve MDR strains isolated from pigs could be classified in to16 and 22 MDR patterns (Table 4). Some common antibiotic resistance patterns were detected in strains of E. caselliiflavus and E. gallinarum (Cd,Co,Os); E. faecalis and S. sanguis (Co,Cp,Os), E. gallinarum and five S. sanguis (Cd,Co,E.Ox); two strains of S. sanguis had the same pattern (Cd,Co,E); two strains of E. coli from two different pigs belonged to each of the two patterns (A,Nf,T; A,Na,C); two strains of E. tarda from two different pigs had the same pattern (A,C,Na,Nf,T). Other common MDR patterns were among strains of C. freundii and E. coli (Ca,Co,Na,F); E. gregoviae and P. aeruginosa (A,C,Na,Co,Ci,Cl,Co,Nf,T). An MDR pattern among E. coli and P. vulgaris (A,Co,Nf) of pig origin was similar to that in E. blattae, K. pneumoniae isolates from house sparrow. Similarly, an MDR pattern (A,Co,Na,Nf) detected in pig E. coli was same to that of K. pneumoniae and Praga fontium of and house sparrow origin. Among G+ve bacteria isolated from pigs MDR was detected in 60% strains.
Resistance was common against cotrimoxazole (90.0%), oxacillin (72.5%), erythromycin (45%) and clindamycin (45%). For other antimicrobial resistance was occasionally detected against ampicillin (15%), cephoxime (2.5%), chloramphenicol (7.5%), gentamicin (10%), tetracycline (2.5%), amoxyccillin+clavulanic acid (10%), cephalaxin (15%), cloxacillin (12.5%) and carbenicillin (10%) while none of the 40 strains was resistant to ciprofloxacin and norfloxacin. All isolates of G+ve bacteria belonging to E. casseliflavus, E. mundatii and E. solitarius had MDR while none of the E. faecium and Micrococcus agilis showed MDR. However, 80% of E. faecalis and E. gallinarum isolates, 50% of E. dispar and 47.6% of Streptococcus sanguis strains had MDR. Oxacillin resistance detected in 72.5% of G+ve bacteria, all E. gallinarum and 80% E. faecalis were resistant to it. Similarly about 80% of E. faecalis strains were resistant to erythromycin. Except E. faecalis (60%) none of the enterococci isolated from pigs was resistant to chloramphenicol. Among enterococci, cephoxime resistance was detected in an E. gallinarum strain only. For most of the antimicrobials, resistance among G+ve bacterial strains of different species not varied significantly (p ¿0.05) except for cephalexin (p = 0.035), chloramphenicol (p = 0.007, skewed for E. faecalis), gentamicin (p = 0.015, skewed for E. faecalis), amoxyccillin+clavulanic acid (p = 0.001, skewed for E. mundatii), cloxacillin (p = 0.009, skewed for E. mundatii and Streptococcus spp.) and carbenicillin (p = 0.021, all 21 streptococci were sensitive). Most of the G-ve bacteria isolated from pigs were resistant to ampicillin (85.2%) and nitrofurantoin (68.5%) while sensitive to gentamicin (90.7%), ciprofloxacin and netillin (88.9%), nalidixic acid (87%), chloramphenicol (85.2%), cotrimoxazole (81.5%), tetracycline (79.6%), cephoxime (79.6%) and cefazidime (66.7%). Majority of the E. coli strains from pigs was resistant to ampicillin (83.5%) and nitrofurantoin (58.8%) and some to cefazidime (29.4%). MDR was detected in only 35.3% of E. coli strains. On the other hand all isolates of A. caviae, Citrobacter freundii, E. gregoviae, K. pneumoniae, P. vulgaris, Pseudomonas aerugiosa and S. enterica from pigs had MDR. Of the 9 E. tarda isolates from 8 pigs, 6 had MDR. Among E. tarda strains resistance to nitrofurantoin and ampicillin was of common occurrence (77.8%) and 44.4% strains were resistant to chloramphenicol, gentamicin, tetracycline and netillin each. Resistance to gentamicin was detected only among E. tarda and A. caviae strains while for netillin resistance was evident in E. tarda (4), E. coli (1) and A. caviae (1) strains. Ciprofloxacin resistant strains belonged to all species of G-ve bacteria but A. caviae, K. pneumoniae and P. vulgaris. 2 test revealed that for most of the antimicrobials resistance pattern was significantly (p = 0.001 to 0.023, mostly due to the more common antimicrobial sensitivity among E. coli strains) different for strains of different species of G-ve strains of pig origin except for ampicillin, tetracycline, nalidixic acid, cefazidime and nitrofurantoin. Although MDR varied among strains of different bacteria of pig and sparrow origin, difference was insignificant among G+ve bacteria (p = 0.54). However, in total the variation was significant (p = 0.06) probably because of the markedly high (p = 0.05) number of resistant strains of G-ve strains prevalent in pigs. Sensitivity of bacterial strains to different antimicrobials varied significantly (p ¡0.001), some drugs were highly effective while other drugs were resisted by majority of the strains.

Table 1. Bacteria isolated from 17 samples of faecal droppings of house sparrow (Passer domesticus) perching in a piggery in Jharnapani, Nagaland.
4. Discussion
In the study more than 45% isolates of bacteria from house sparrow in a tribal state of Nagaland where antimicrobial use is minimum had MDR which is surprising and of public health and environmental safety concern. In recent years, the population of house sparrow (Passer domesticus) is down by almost 75% and it might be due to their susceptibility to emerging enteropathogens causing mortality in these birds (8, 26). The observation revealed that out of 34 enterococci isolated from birds 9 were resistant to amoxyclav, i.e., might be producing clavulanic acid resistant \(-\)lactamases (27, 28). Along with amoxyclav resistance, resistance to cephotaxime/ oxacillin/ cloxacillin in enterococci of sparrow origin is
indicative of production of extended spectrum -
lactamases (ESBLs) by the isolates (29, 30) which
is of public health concern as birds are reported to
disseminate bacteria to human and animals (11, 31).
Although, isolates of enterococci resistant to amoxyclav+ oxacillin/ cloxacillin from pigs
belonged to different species, their comparable
ability to produce ESBLs indicated the prevale-
ce and circulation of similar gene cascades
responsible for emerging antimicrobial resistance.
From diarrhoeic pigs, E. coli was the most pre-
dominant G-ve bacteria isolated in pure as well as
in mixed infection state from 26 rectal swabs fol-
lowed by E. tarda (9), both of the pathogens have
been documented earlier as the common cause of
diarrhoea in pigs in Northern India too (32, 33)
and abroad (18, 19). Other occasional causes of
diarrhoea in pigs including Salmonella enterica,
Aeromonas caviae, pseudomonads, streptococci
and enterococci were also evidently isolated as
dause of diarrhoea in the present study from a
few rectal swabs either as pure or in mixed infec-
tion state as reported in earlier studies (20, 21).
No clear association between bacterial isolates of
house sparrow and diarrhoeic pigs could be estab-
lished in the present investigation. From earlier
studies (4, 5, 10-12), it is not clear either the
birds or the humans/ animals were the primary
source, but reports are there that several com-
mon pathogens, often opportunistic ones, were
isolated from diverse sources. Birds, particularly
those live in or near human populated areas are
considered biologically very important due to
their potential for transport and dissemination of
pathogens (31). Birds may be involved in dispersal
of microorganisms either as biological carriers,
or mechanical carriers. Many species of microbes
pathogenic to domesticated animals and birds and
also to human beings have been associated with
free-living birds (31). In the present investigation
too, several bacteria of zoonotic potential were
isolated from droppings of house sparrow as well
as from pig rectal swabs. However, there was sig-
ificant difference in occurrence of different type
of bacteria in birds and pigs (p 0.001). Among
G-ve bacteria isolated from birds only 59% of the
isolates belonged to the species (C. freundii, E.
coli, Enterobacter gregoviae and K. pneumoniae)
also isolated from the pigs while 72.2% isolates
of pig origin belonged to such common species.
There were 5 species (A. caviae, Edwardsiella
tarda, Proteus vulgaris, Pseudomonas aeruginosa
and Salmonella enterica) of G-ve bacteria solely
isolated from diarrhoeic pigs while strains of 10
species (Escherichia fergusonii, Escherichia blatt-
tae, Enterobacter agglomerans, Klebsiella planti-
cola, Praga fontium, Proteus mirabilis, Serratia
fiscaria, Serratia odorifera, Serratia plymuthica
d Xinorhabdus bovienii) were only detected in
birds only. Similarity among G+ve strains was
still less, only 44.8% strains of bird origin belong
to those two species detected in pigs also (E. fae-
calci and E. gallinarum) while from pigs only 25%
were such strains. Majority of G+ve bacteria iso-
lated from birds and pigs belonged to dissimilar
species as strains of E. casseliflavus, E. dispar, E.
faecium, E. mundatii, E. solitarius, Micrococcus
agilis, and Streptococcus sanguis species were iso-
lated from pigs only while those of E. avium and
E. caecorum species isolates from birds only. This
may be due to the difference in commensal mi-
crobiome, digestive physiology and feeding habits
of the two (11, 31). In contrast to enterococci
of birds which were more resistant to multiple
drugs than those isolated from pigs; significantly
more numbers of the G-ve bacteria isolated from
pigs were resistant to either cephphotaxime or ceft-
taxidime or both (20/54) than from birds (6/34)
indicating the higher prevalence of ESBLs produc-
ing (27-30) potentially zoonotic enteropathogens
in pigs. Resistance pattern of strains of almost all
different species of enteric bacteria isolated from
pigs including Salmonella enterica ssp. enterica,
E. coli, K. pneumonia, E. tarda, Pseudomonas
aeruginosa and Proteus vulgaris indicated poten-
tial of possessing ESBLs while none of the isolate
from sparrow was resistant to cephphotaxime and
only few (6) to cfxtazidime indicating less possi-
bility of circulation of G-ve bacteria among birds
and pigs residing together. Further, role of birds
in dispersion of enteric bacteria causing diarrhoea
in pig did not seem to be very important because
among the enteric bacteria isolated from pig as
well as birds dissimilarity in their antibiogram
was very much evident (Table 2 and 4). None of
the strain belonging to the same species isolated
from house sparrow and pig samples had similar
antibiotic sensitivity pattern, indicating the dif-
ference among the strains of the same species of
different origin. However, there was similarity in
MDR pattern among strains of different species
isolated from pig and birds viz., E. coli and P.
vulgaris (A,Co,Nf) of pig origin had MDR as of
E. blattae and K. pneumoniae from house spar-
row; an MDR pattern (A,Co,Na,Nf) of pig E. coli
was same to that of K. pneumoniae and Praga
fontium of sparrow origin. The idea of circulation
of common bacteria in pigs and birds perching
in pig shed was further countered by the obser-
vation by the fact that many of the pathogens
associated with diarrhoea in pigs were not de-
ected from samples of birds viz., Salmonella enterica, Aeromonas caviae, Edwardsiella tarda and Enterococcus solitarius. The similarity of anti-biogram in strains of different species of bacteria and also of different origin indicated that either the similar or the same resistant genes were circulating among bacterial populations under varying environment i.e., bacteria may not be zoonotic or spreading as frequently as the resistant genes. Although Salmonella (34-37), Escherichia coli (35, 38, 39), Enterococcus faecalis (40), have been documented to spread through birds, the isolates in the present study from dirahoeic pigs and birds had no or little similarity. Reasons behind the dissimilarity of the bacterial strains isolated from co-habited house sparrow and pigs may be associated with several biotic and abiotic attributes associated with survival and dispersal of the microbe (11, 31).

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References


