Migratory bird, *Branta leucopis* (Barnacle goose), a potential carrier of diverse *Escherichia coli* serotypes into pristine Arctic environment

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As a part of the Indian summer expedition to the Arctic in 2011, a study was carried out on the diversity and antibiotic resistance of *Escherichia coli* serotypes in the droppings of migratory bird *Branta leucopis*, widely known as Barnacle goose. Serotyping of 41 different isolates of *E. coli* from the droppings revealed presence of nine different serotypes dominated by O149 and O24. The other serotypes encountered were O148, O2, O21, O91, O130, O32 and O37. Phylogenetic analysis by triplex PCR revealed that 31.7% of the isolates belonged to group B2, which are the most virulent strains, followed by B1 and A groups which are considered as commensal strains. Less virulent D group strains were nearly 10%. Antibiogram of the isolates revealed that all the strains were resistant to colistin (polymixin E). While there was a modest level of beta lactam resistance (ampicillin – 39%; amoxicillin – 12%) among the *E. coli* isolates from this source, 7% was resistant to tetracycline.

Keywords: Antibiotic resistance, barnacle goose, *Escherichia coli*, migratory birds, serotyping.

MIGRATORY birds are gaining increasing relevance as a means of spreading multidrug-resistant pathogens across geographical boundaries[1]. During their often long migratory flights, these birds have recovery points where they intermingle with the local population of birds, feed and pick up strains of bacteria that are of public health significance. These could be then disseminated to pristine environments such as the Arctic and the Antarctic. Barnacle goose (*Branta leucopis*) breeds mainly in the arctic islands of North Atlantic and includes three main populations with separate breeding and wintering ranges. The population of barnacle goose considered here is from Svalbard islands, which breeds in Svalbard with wintering on the Solway Firth on the England/Scotland border. This is a typical migratory bird which travels between extremely localized breeding and wintering areas[2]. The migratory flight includes regular stop-over sites and uses the same nesting sites year after year. These habits make it an ideal choice to study the association of bacteria of public health significance with them and possible dissemination into the pristine Arctic environment. *Escherichia coli*, though widely considered as a commensal bacterium, is gaining increasing relevance and acquiring the status of an emerging pathogen. Its widespread presence in nature and frequent horizontal gene transfer in the environment helps it to gain properties such as virulence and multiple drug resistance and emerge as a pathogen of significance[3].

India has opened its permanent Arctic research station 'Himadri' at the International Arctic Research Centre at Ny-Alesund, Svalbard, Norway in 2008 and since then has been engaged in various research activities. The Svalbard region (79°58′N) is considered as relatively pristine and offers good feeding and breeding ground for Barnacle goose, which is a prominent bird population here and could act as a means of dissemination of potentially pathogenic bacteria into this environment. Though there are reports of dissemination of antibiotic-resistant bacteria across geographic borders[4] and antibiotic resistance in Enterobacteriaceae isolated from wild birds[5], specific reports from bird communities in Svalbard are not available. Hence during the Indian Arctic expedition in summer 2011, a study was undertaken on the diversity and antibiotic resistance of *E. coli* in the droppings of migratory bird *B. leucopis*.

Fresh droppings (30 samples) of *B. leucopis* were collected during July 2011 from the feeding areas in Ny-Alesund region of Svalbard. Samples were collected individually in sterile polythene bags and brought to the Kingsbay marine laboratory at the International Arctic Research Station within 1 h of collection and processed for isolation of *E. coli*. One gram of dropping was inoculated into 9 ml of normal strength lactose broth and incubated at 37°C for 24 h. The samples showing growth and gas production were streaked on eosin methylene blue agar (EMB, HiMedia) and incubated at 37°C for 24 h. After incubation, the plates were observed for typical *E. coli*-like colonies and whenever present were isolated onto sterile nutrient agar slants. These were restreaked on EMB agar plates to ensure purity and maintained on nutrient agar vials. Biochemical characterization of the *E. coli* strains was carried out based on indole, methyl red, Voges–Proskauer and citrate (IMViC) tests.

Table 1. Phylogenetic groups of *Escherichia coli* and occurrence pattern of genes involved

Phylogenetic group	Expected gene profile
Group B2	*ChuA*$^+$, *YjaA*$^+$/*ChuA*$^+$, *YjaA*$^+$, TspE4.C2$^+$
Group D	*ChuA*$^+$, *YjaA*$^-$/*ChuA*$^+$, TspE4.C2$^+$
Group B1	*ChuA*$^-$, *TspE4.C2*$^+$/*YjaA*$^+$, TspE4.C2$^+$
Group A	*ChuA*$^-$, *TspE4.C2*$^-$/*ChuA*$^-$, *YjaA*$^+$, TspE4.C2$^-$

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Table 2. Percentage occurrence of various serotypes and phylogenetic groups of *E. coli* in the droppings of *Branta leucopis*

Serotype of *E. coli*	Percentage of incidence	Phylotype of *E. coli*	Percentage of incidence
O149	24.39	A	17.07
O24	17.07	B1	41.46
O148	12.17	B2	31.7
O2	9.75	D	9.75
O21	2.43		
O91	2.43		
O130	2.43		
O32	2.43		
O37	2.43		
Rough/untypable	24.39		

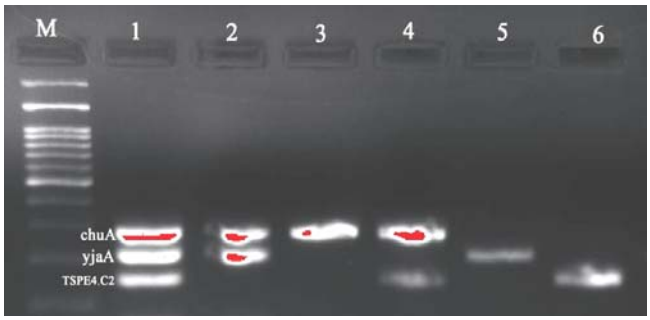

Figure 1. Gel showing amplification of *chuA*, *yjaA* and *TSPE4.C2* genes by triplex PCR.

Confirmed isolates were serotyped at the National *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh.

The phylogenetic group was determined by a triplex PCR method as described previously[6] (Table 1). The primers used were ChuA.1 (5′-GACGAACCAACGGTCAGGAT-3′) and ChuA.2 (5′-TGCCGCCAGTACCAAAGACA-3′), YjaA.1 (5′-TGAAGTGTCAGGAGACGCTG-3′) and YjaA.2 (5′-ATGGAGAATGCGTTCCTCAAC-3′), TspE4C2.1 (5′-GAGTAATGTCGGGGCATTCA-3′) and TspE4C2.2 (5′-CGCGCCAACAAAGTATTACG-3′). This PCR is based on the amplification of two genes (*chuA* and *yjaA*) and one genomic fragment (TSPE4.C2). The optimized protocol was carried out with a PCR mix of 20 µl containing 1.5 mM $MgCl_2$, 2.5 µl *Taq* buffer [(Tris (pH 9.0) at 25°C, KCl and Triton X-100)], 2 mM each of dNTP mixture, 20 pmol each of the primers, 2.5 U of *Taq* polymerase (GeNei™, India) and 1 µg of DNA template. The amplification consisted of the following steps: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation (30 sec at 94°C), annealing (30 sec at 55°C) and extension (30 sec at 72°C), and a final extension step of 7 min at 72°C. PCR products were then electrophoresed on a 1.5% agarose gel (HiMedia, India), stained with ethidium bromide (GeNei™, India) and visualized by Gel Documentation System (BioRad Gel Doc™ EZ Imager, USA).

Antibiograms and their interpretation were made using the disk diffusion method[7], following the Clinical and Laboratory Standards Institute (CLSI) guidelines[8]. All *E. coli* isolates were examined for resistance to ampicillin (Amp, 10 mcg), amoxycillin (Amx, 30 mcg), ceftazidime (Cat, 30 mcg), chloramphenicol (C, 30 mcg), ciprofloxacin (Cip, 5 mcg), colistin (Cl, 10 mcg), co-trimoxazole (Cot, 25 mcg), gentamicin (Gen, 10 mcg), nalidixic acid (Na, 30 mcg), streptomycin (S, 10 mcg) and tetracycline (Te, 30 mcg). All the antibiotic disks used were from HiMedia.

All the samples yielded positive isolation of *E. coli*. Serotyping of the strains revealed eight different serotypes; O149 (24.39%) was predominant whereas O24 (17.07%) and O148 (12.17%) were frequently encountered in the droppings of *B. leucopis* (Table 2). Phylogenetic analyses have shown that *E. coli* strains fall into four main phylogenetic groups A, B1, B2 and D (Figures 1 and 2). Dissemination of multidrug bacteria by Arctic birds into Siberia and Greenland regions of the Arctic[4] and antibiotic resistance among Enterobacteriaceae from wild birds such as gulls from North West Europe[9] have been reported. However, there are no reports on the phylotypes of *E. coli* in *B. leucopis* from Svalbard region. Our results highlight diversity of pathogenic phylotypes of *E. coli* in the droppings of this bird population. Most virulent extraintestinal strains belong to group B2 or less frequently, group D, whereas most commensal strains belong to groups A and B1 (ref. 10).

Antibiogram of the *E. coli* strains revealed modest levels of resistance against beta lactam antibiotics such as ampicillin (39%) and amoxicillin (12%). The least resistance was detected against tetracycline (7%) and ceftazidime (2%). All the strains were sensitive to antibiotics such as ciprofloxacin, gentamicin, co-trimoxazole, nalidixic acid, streptomycin and chloramphenicol, indicating lack of selection pressure against these antibiotics. Drug resistance among the microbiota of Arctic birds was explained by various researchers as *de novo* development through spontaneous mutations[11], horizontal gene transfer among natural reservoirs and bird microbiota[12], and

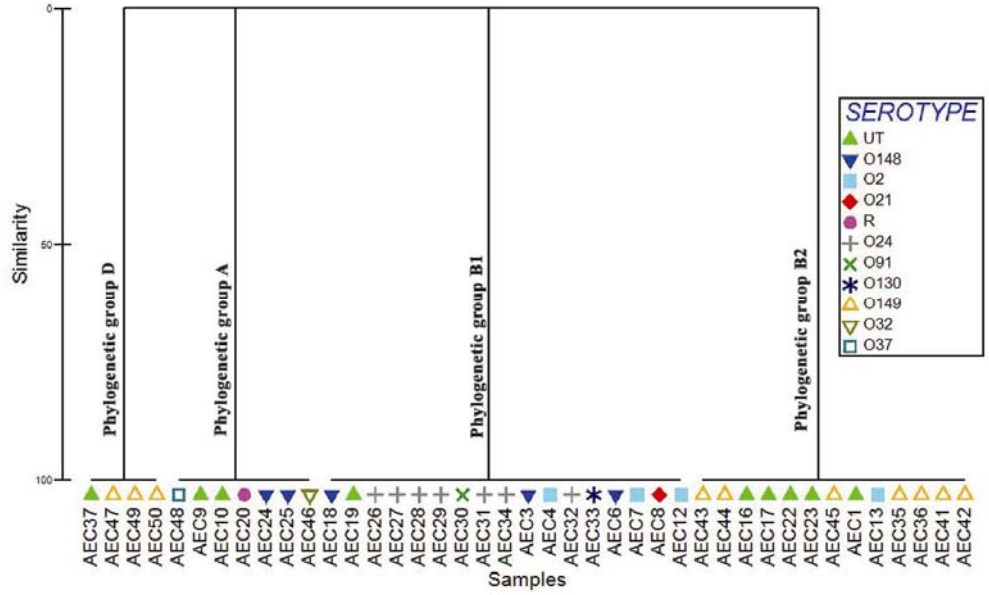

Figure 2. Phylogenetic tree of *Escherichia coli* serotypes isolated from *Branta leucopis*.

deposition of resistant strains through migratory birds[5]. Some of the wintering areas of migratory birds are frequented by bird populations from six different continents[13]. Lack of antibiotic resistance among bacteria from wild bird populations without interaction with humans throughout the year[6] indicates that antibiotic resistance genes picked up by migratory birds at recovery points and wintering areas are the source of antibiotic resistance genes in pristine environments such as the Arctic. An interesting finding of our study was the high prevalence of colistin (polymixin E) resistance among *E. coli* from *B. leucopis*. This antibiotic was sparingly used in the past due to its reported nephrotoxicity and neurotoxicity, and the resistance levels are quite low. However, there is a recent revival in the use of colistin for the management of multidrug-resistant Gram-negative infections[14], as the potential effectiveness outweighs the toxicity reported in previous studies. It would be interesting in the envisaged follow-up studies on microbial source tracking, to look for the selection pressure for this resistant gene. The present study highlights the role of *B. leucopis* as a possible means of dissemination of diverse *E. coli* serotypes into the Svalbard region of the Arctic.

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