to overcome varied ailments including worm disease condition, stomach-ache, jaundice and malaria like fevers. This study is an attempt to assess AC as antimicrobial and antimalarial plant. Therefore, crude extract and fractions from AC nuts were tested against *Plasmodium falciparum* 3D7 and 11 strains of bacteria.

Methods & Materials: Extract from AC nuts was obtained by Sohlet extraction using methanol as solvent. Fractions viz. hexane, chloroform, ethyacetate, butanol and water were obtained by partitioning methanol extract in water and different solvents in order of increased polarity. Antimalarial activity of extract and fractions from AC was performed through SYBR green method whilst antimicrobial activity was assessed through Disc Diffusion Assay (DDA). The antimicrobial potential was further confirmed quantitatively by determination of the Minimum Inhibitory Concentration (MIC) of extract and fractions from AC.

Results: Extract and fractions from AC showed remarkable antimalarial activity against *P. falciparum*, the most potent being butanol fraction with an IC_{50} of 18 μg/ml. Significant antimicrobial activity of methanol extract, ethylacetate, butanol and water fraction towards four bacterial strains viz. *Staphylococcus Aureus* 96 (SA96), *Staphylococcus Aureus* 2940 (SA2940), *Streptococcus mutans* (SM) and *Mycobacterium smegmatis* (MS) was recorded. The most potent being butanol fraction with DDA of 14 mm. The MIC values were 125, 250, 62.5 and 250 μg/ml for methanol extract, ethylacetate, butanol and water fractions towards SA96.

Conclusion: AC demonstrated to possess antimalarial and broad antimicrobial activities which might be prospected as potential source for the development of new therapeutic agents.

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Proline residue at $NS3_{249}$ is a primary determinant of West Nile virus virulence in mammals and birds

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Background: West Nile virus (WNV) is a neurotropic flavivirus transmitted through mosquito bites and whose reservoir hosts are wild birds. Equids and humans are incidental dead end hosts and can develop severe neurological symptoms. Despite enhanced reporting of WNV outbreaks in Europe since 2008, much remains to be explored about the virulence level and determinants of WNV strains circulating in Europe. Of note, recently expanding lineage 2 virus has gained a proline residue at position 249 in the non structural 3 (NS3) protein and has caused the worst epidemics ever experienced in central and Southern Europe. Interestingly, Brault *et al.* (2007) identified $NS3_{249P}$ as being crucial for the virulence of WNV in American crows.

Methods & Materials: An infectious clone, based on WNV lineage 1 IS-98-ST1, a highly neuroinvasive strain, harbouring $NS3_{249P}$, was constructed (Bahuon *et al.*, 2012) and a $NS3_{P249T}$ mutant was generated by directed mutagenesis.

We aimed at deciphering the properties of recombinant viral particles *in vitro* and *in vivo*, in mammalian and bird models (Dridi *et al.*, 2013).

Results: In Vero cells, virus with a $NS3_{249T}$ protein proved to replicate at a slower rate than the parental $NS3_{249P}$ virus. When injected intraperitoneally in adult female Balbc/J mice, parental virus was found to be highly virulent (LD50 < 1 pfu), while only 4 out of 20 animals infected with the $NS3_{249T}$ virus succumbed, regardless of the initial infecting dose (1-10^3 pfu). Mice infected with $NS3_{249T}$ virus experienced milder clinical and virological outcomes, characterized by delayed weight loss and decreased viremia 4 days pi ($1.4x10^3$ vs $3.2x10^4$ viral copies/mL blood). Birds, one-day old chicks and young corvids (*Corvus corone*), also indicated that $NS3_{249T}$ virus was attenuated for model and susceptible European birds. In particular, in young crows, 16.7% (1/6) animals died after subcutaneous infection with $NS3_{249T}$ virus whereas a 100% lethality (7/7) was observed with $NS3_{249P}$ virus.

Conclusion: The presence of a proline residue at position 249 in NS3 appears as a primary determinant for WNV virulence in wild birds, as well as in mammals and could be a genetic factor accounting for enhanced reporting of WNV neuro-invasive cases in humans in Europe.

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Genetic Diversity of West Nile virus Isolated from the tick, Rhipicephalus pulchellus, in Kenya

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Background: West Nile virus is a re-emerging infectious disease that has a wide geographical distribution in Africa, parts of Europe, the Middle East, Asia, Australia and the Americas where it causes outbreaks. Although WNV has been isolated in mosquitoes in Kenya, paucity of genetic information exists. Mosquitoes are the traditional vectors for WNV however; the virus has also been isolated from some tick species in North Africa and Europe which could be a means of introduction and spreading of the virus over long distances through migratory birds. North-Eastern province of Kenya has been found to be a major hot spot for arbovirus circulation where arboviruses such as Rift Valley fever (RVFV), WNV and Crimean-Congo hemorrhagic fever have been isolated from vectors and humans. Moreover, some of these viruses such as RVF has been reported to have caused outbreaks in the province.

Methods & Materials: In this study ticks were sampled from restrained animal hosts (livestock and wildlife), classified to species and processed in pools of up to 8 ticks per pool. Virus screening was performed by cell culture using Vero cells and RT-PCR. Positive cases were subjected to 454 sequencing. Phylogenetic analysis was carried out to determine the evolutionary relationships of our isolates.

Results: Among other viruses, WNV was isolated from a pool of *Rhipicephalus pulchellus.* Sequence data is available in gene bank and this forms the focus of this report. Comparative analysis with 27 different WNV strains from other regions revealed that our isolate belongs to lineage 1.

Conclusion: Overall, phylogenetic analysis based on nucleotide sequences showed that although genetically distinct, the WNV strain obtained in this study clustered relatively closely to virus isolates from Russia, Europe and the United States belonging to lineage 1 of WNV. To our knowledge, this is the first documented isolation of WNV from *Rhipicephalus pulchellus*. It is plausible that wild migratory birds may have dispersed these lineages among these continents through tick vectors.

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Circulation, evolution and transmission of ngari and bunyamwera orthobunya viruses in Northern Kenya

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Background: Although genetic drift/reassortment seems to occur frequently in the Bunyaviridae family, the epidemiological consequences of these evolutionary events are poorly understood. Moreover, these viruses have been isolated from a range of mosquito species but the actual role of the associated mosquito species in the maintenance, transmission and evolution of the virus in the environment remains unclear. Our objective was to understand the dynamics of circulation, reassortment and transmission of Bunyamwera and Ngari viruses in Kenya.

Methods & Materials: Selected Bunyamwera and Ngari virus isolates from previous surveillance exercises in Kenya were cultured in Vero cells to prepare viral stocks. Total RNA was isolated from culture and specific primers designed against the genome segments using Bunyamwera/Batai virus sequences in Genebank. Sequencing was performed in ABI 3700 genetic analyzer and phylogenetic analysis using MEGA v5.2. Sequences were compared with selected members of the Orthobunyavirus genus isolated from different geographical locations. For vector competence, female mosquitoes were exposed to blood-virus mixture (10^{10} *Pfu/ml*). Engorged mosquitoes were transferred to clean cages and maintained with sucrose solution at 28°C for 14 days. A sub-set of experimental mosquitoes were killed by freezing at days 7 and 14 and virus assayed in both legs and body separately by plaque assay.

Results: We identified isolates of both viruses which were relatively conserved regardless of the species or region of isolation with the Kenyan Ngari isolates clustering closest with the Ngari strains associated with the 1997-1998 hemorrhagic fever outbreaks in East Africa. *Aedes aegypti* was refractory to Ngari virus but susceptible to Bunyamwera virus and could be a competent vector for Bunyamwera virus. *Anopheles gambiae* was susceptible to both Ngari and Bunyamwera viruses but lower dissemination rates for the later. This may have been possibly due to genetic reassortment and warrants further investigation. This has major implication in light of continued animal trade and travel especially into malaria endemic regions where *Anopheles gambiae* is more prevalent.

Conclusion: Our results underscore the need to continually monitor emergent arboviral genotypes circulating within particular regions as well as vectors mediating these transmissions in order to preempt and prevent their adverse effects.

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Predictors of nosocomial *Clostridium difficile* in infectious disease hospital

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Background: In our hospital, an 500 beds infection diseases clinic, admissions of cases of *Clostridium difficile* enteritis (CDE) commenced to increase consistently starting with mid of 2011; in response to this contact precautions were implemented and healthcare workers were trained but these interventions didn't totally prevented intrahospital transmission of spores and appearance of nosocomial *Clostridium difficile* enteritis.

Methods & Materials: We attempted to discover predictors for nosocomial CDE in our hospital

We conducted a retrospective chart review of all patients consecutively discharged from hospital between August 2011 and July 2012, if one of the final diagnosis was *Clostridium difficile* enteritis (Code ICD – 10: A04.7). Data collected and entered in an Epi Info data base were: age, gender, duration of hospital stays, outcome, ICU residence and Charlson comorbidy index (CCI). A case of nosocomial CDE was defined as a patient with onset of diarrheic (3 3 unformed stools per day) after 48 hours from hospital admission and with a positive test for *Clostridium difficile* toxin.

Results: During the selected time interval the prevalence of nosocomial CDE was 18,8% (37/197). Univariate analysis: (a) excluded as risk factors ($p > 0.05$) the following variables: age > 70 years, the male gender and a CCI > 6 and (b) retained as risk factors the followings: a longer ($\geq$ 17 days) stay in hospital ($p < 0001$), a fatal outcome ($p < 0.005$) and an installment in ICU ($p < 0.0001$). Later on the logistic regression revealed as risk factors independently associated (predictors) to nosocomial CDE only two variables: longer hospital stay (OR: 4.31; 95%CI: 1.87 – 9.89; $p = 0.0006$) and ICU residence (OR: 4.70; 95%CI: 1.76 – 12.55; $p = 0.0020$.)