Neisseria gonorrhoeae multiantigen sequence typing (NG-MAST) of isolates collected from STD patients in Delhi, India

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Background

- *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST) is a highly discriminatory technique for assessing the genetic diversity of clinical isolates
- It has also been put forward as a tool for predicting AMR phenotypes
- The distribution of NG-MAST sequence types (STs) in *N. gonorrhoeae* isolates differs between isolates from different geographical regions
- Therefore, the present study was undertaken to determine the NG-MAST genotype distribution of isolates in Delhi and to examine if it can be used as a means for predicting AMR
- This is in compliance with the WHO recommendations that emphasize on research into newer molecular methods for monitoring & detecting AMR in *N. gonorrhoeae*
- This is first such research performed in our country

Methods

- A total of 100 *N. gonorrhoeae* consecutive isolates (99 from male & 1 from female patients) collected between April 2010 to October 2013 were investigated
- The age group of infected patients ranged from 17 to 54 years; median age 27 years
- *N. gonorrhoeae* culture and identification was done as per standard methods
- The antibiotic susceptibility testing (AST) was performed by the CDS technique with low concentration discs and MICs of penicillin, ciprofloxacin, tetracycline, ceftriaxone, azithromycin & spectinomycin for all isolates was determined by Etest (Biomerieux, France)
- All isolates were tested for β lactamase production by nitrocefin discs (BD, USA)
- NG-MAST was performed on all isolates as previously described (Martin et al, 2004)
- WHO *N. gonorrhoeae* reference strains F, G, K- P were used as controls
- Phylogenetic analysis was done by Maximum Likelihood method using MEGA6
- Association between NG-MAST sequence type & antibiotic phenotypes was probed using chi-square & fisher's exact tests
- All statistical analysis were done by using STATA 11.2 and results were considered as significant at p<0.05

Results

- Penicillin: The percentage of resistant isolates varied insignificantly from 2010 to 2013 (χ^2=5.16, p=0.164). This includes PPNG (penicillinase producing *N. gonorrhoeae*) & CMRNG (chromosomally-mediated resistance *N. gonorrhoeae*)
- Tetracycline: Resistance to tetracycline increased significantly from 2010 to 2013 (χ^2=14.08, p=0.016). It includes plasmid-mediated tetracycline resistant *N. gonorrhoeae* (TRNG) & chromosomally-mediated resistance (CMR)
- The rate of resistance to ciprofloxacin were overall very high (94%) & showed a significantly increasing trend from 2010 to 2013 (χ^2=12.02, p=0.042)
- Overall Decreased susceptibility (DS) to ceftriaxone was demonstrated in 5% of isolates that were restricted to years 2010 & 2011. No isolates demonstrated DS in 2012 & 2013
- Azithromycin: Overall resistance was seen in 4% of isolates with no significant trends
- All isolates were susceptible to spectinomycin & No MDR-NG or XDR-NG strains was observed
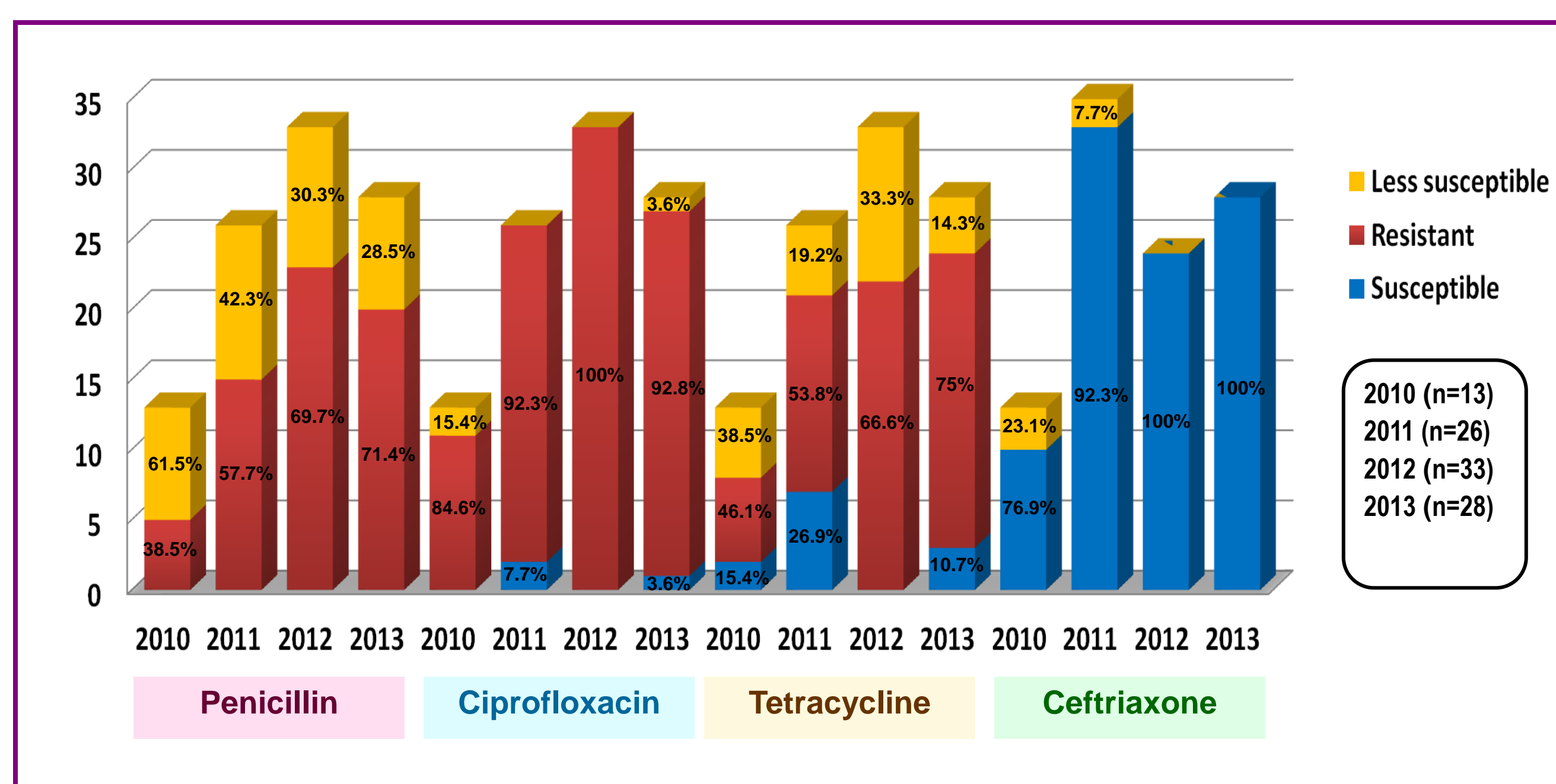

Figure 1: Antimicrobial susceptibility profile of *Neisseria gonorrhoeae* isolates (n=100) to the 4 antimicrobials from 2010 to 2013

NG-MAST

- *N. gonorrhoeae* isolates were distributed among 60 different STs, 43 (71.6%) of which have not been reported previously to the international database
- The majority of STs (76.6%, 46 of 60) were represented by singletons
- The most common STs were ST6058 (n=21, 21%), followed by ST9774 (n=4, 4%), ST9875 (n=4, 4%), ST9783 (n=4, 4%) and ST2990 (n=3, 3%)
- Our DS isolates belongs to ST6069 (n=2), ST6083, ST8156, ST9820 (one each respectively)
- There was significant association between ST6058 & resistance to penicillin (p=0.00) & tetracycline (p=0.002)
- In all the other antibiotics, no association was found

Phylogenetic analysis

- The high genetic diversity arose from the allelic combination of 48 *por* and 31 *tbpB* alleles
- The overall mean genetic distance was 90.4 (SE 4.6) nucleotide differences, where a maximum distance of 280 (SE 12.1) nucleotides was observed between ST9878 and ST621
- The 60 STs formed three groups separated by (Group A - Group B) 121.6 (SE 8.7), (Group B - Group C) 239 (SE 11.4) and (Group A – Group C) 222.3 (SE 11.2) nucleotide differences
- The mean genetic distance within group A is 44.5 (SE 3.7), within group B is 39.4 (SE 3.6) & within group C is 36.3 (SE 4.7) nucleotides
- The largest number of isolates were seen in Group A that also included the most prevalent ST6058 & DS strains
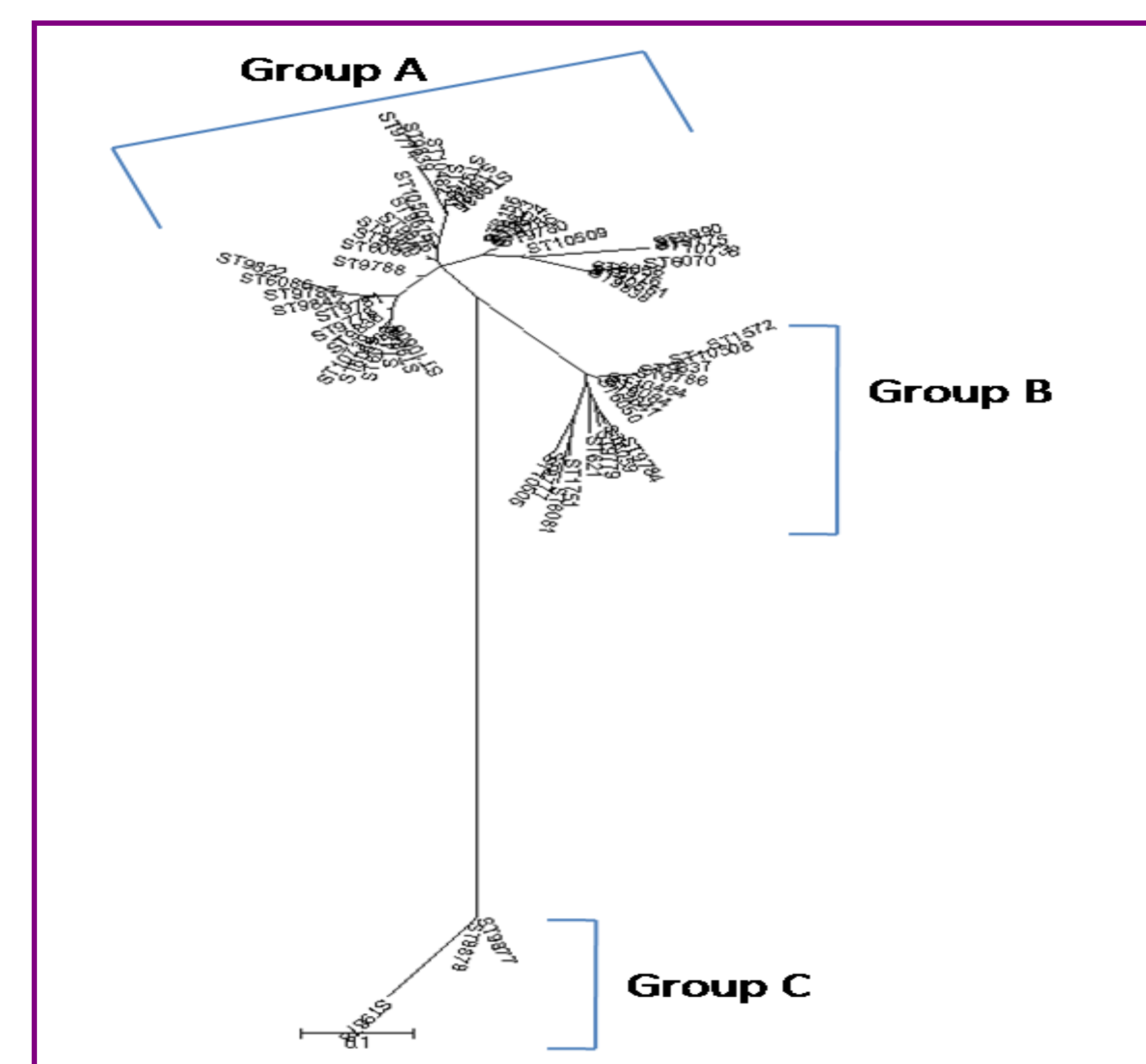

Figure 2: Unrooted maximum likelihood tree for all the NG-MAST sequence types identified in Delhi based on distance estimates using the number of nucleotide differences model

Discussion & Conclusion

- The rates of resistance to traditional antibiotics were high
- Although decreased susceptibility (DS) to injectable ceftriaxone was seen in 5% isolates, there was no treatment failure reported
- There is need to monitor MICs of ceftriaxone as the percentage of strains showing 2 fold increase in modal values increased from 38% in 2010 to 57% in 2013
- However, our data fully supports the currently recommended combination of ceftriaxone & azithromycin for treatment of gonorrhoea
- The phylogenetic tree demonstrated a large strain diversity in our gonococcal population
- Like other authors, we too feel that it is because of local emergence of new STs and import of foreign STs
- The most common ST was ST6058, seen in 21% of isolates
- ST1407, the internationally documented multidrug resistant gonococcal clone was not seen
- Although there was a significant association between resistance to penicillin & tetracycline and ST6058, our results reflect a limited applicability of NG-MAST as a tool for predicting AMR phenotypes in our region
- There is need to access the value of this technique in a large number of isolates and also to evaluate alternative technologies